"A RANDOMIZED CLINICAL TRIAL TO EVALUATE THE EFFICACY OF ARIMEDADI OIL PULLING IN PLAQUE INDUCED GINGIVITIS AND ITS EFFECT ON METABOLIC MARKER IN GINGIVAL CREVICULAR FLUID".

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In partial fulfillment for the Degree of

MASTER OF DENTAL SURGERY

BRANCH II
DEPARTMENT OF PERIODONTICS

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CERTIFICATE

This is to certify that this dissertation titled **"A RANDOMIZED CLINICAL TRIAL TO EVALUATE THE EFFICACY OF ARIMEDADI OIL PULLING IN PLAQUE INDUCED GINGIVITIS AND ITS EFFECT ON METABOLIC MARKER IN GINGIVAL CREVICULAR FLUID"** is a bonafide record of work done by **Dr. SHANMUGA SUNDARI S**, under our guidance and to our satisfaction, during her postgraduate study period of 2013-2016.

This dissertation is submitted to **THE TAMILNADU DR. MGR MEDICAL UNIVERSITY** in partial fulfilment for the award of the degree of **MASTER OF DENTAL SURGERY - PERIODONTICS, BRANCH II**. It has not been submitted (partial or full) for the award of any other degree or diploma.

Dr. Koshy Chithresan, MDS,
Professor & Head of the Department,
Department of Periodontics,
Sri Ramakrishna Dental College
and Hospital, Coimbatore.

Dr. Arun Maradi, MDS,
Reader & Guide,
Department of periodontics ,
Sri Ramakrishna dental college
and Hospital, Coimbatore.

Dr. V. Prabhakar, MDS,
Principal,
Sri Ramakrishna Dental College
and Hospital,Coimbatore.

Date:

Place: Coimbatore.

DECLARATION

TITLE OF DISSERTATION	**"A RANDOMIZED CLINICAL TRIAL TO EVALUATE THE EFFICACY OF ARIMEDADI OIL PULLING IN PLAQUE INDUCED GINGIVITIS AND ITS EFFECT ON METABOLIC MARKER IN GINGIVAL CREVICULAR FLUID".**
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DURATION OF THE COURSE	**3 YEARS**
HEAD OF THE DEPARTMENT	**DR. KOSHY CHITHRESAN**
NAME OF THE GUIDE	**DR. ARUN MARADI**

I hereby declare that no part of the dissertation will be utilized for gaining financial assistance/any promotion without obtaining prior permission of the Principal, Sri Ramakrishna Dental College and Hospital, Coimbatore. In addition, I declare that no part of this work will be published either in print or in electronic media without the permission of the guide who has been actively involved in dissertation. The author has the right to reserve for publish of work solely with the prior permission of the Principal, Sri Ramakrishna Dental College and Hospital, Coimbatore.

Head of the Department **PG Guide** **Signature of the Candidate**

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"The best teacher is not the one who knows most but the one who is most capable of reducing knowledge to that simple compound of the obvious and wonderful."

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S. SHANMUGA SUNDARI

ABSTRACT

TITLE: A randomized clinical trial to evaluate the efficacy of Arimedadi oil pulling in plaque induced gingivitis, and its effect on metabolic marker in gingival crevicular fluid.

BACKGROUND: In the oral cavity, plaque forms biofilm/pellicle over the tooth surface, that harbours numerous microorganism. These microorganisms cause inflammation of the gingiva, which is termed as gingivitis. The changes noticed are red, swollen, spongy and bleeding gums. No studies till date have taken place to scientifically prove the efficacy of kabala (medicated oil pulling) in periodontal conditions. Arimedadi tailam, classical ayurvedic formulation is indicated in mukha rogas (diseases of face and oral cavity) in general and specific, oral conditions, danta vidradhi (alveolar abscess), seetada (spongy or bleeding gums), dantaharsha (odontitis), krimidanta (caries), dalana (tooth ache) and the like.

AIM: To evaluate the efficacy of Arimedadi oil pulling (which is a medicated oil containing Ayurvedic medicaments) in plaque induced gingivitis and to assess the clinical and biochemical changes that take place. The patients are randomly selected. Clinical and biochemical parameters are assessed at baseline, II week and IV week.

MATERIALS AND METHODS: Twenty nine patients who were diagnosed with gingivitis were advised to practice Oil pulling therapy or Chlorhexidine rinsing or Placebo mouthrinsing containing mint flavored distilled water for a period of 30 days. The baseline and post-intervention (II week and IV week) - Gingival Index, Plaque Index, Modified Sulcular Bleeding Index and GCF PGE_2 levels were assessed to quantify the statistical difference between these two groups.

RESULTS: Significant reduction was observed between the baseline and post-intervention (II week and IV week) Gingival Index, Plaque Index, Bleeding scores and GCF PGE_2 levels in the chlorhexidine group and Oil pulling group. However, this reduction was greater in Chlorhexidine group than oil pulling group. Overall from baseline to II week and IV week, when the reduction in PlI, GI, BOP and GCF PGE_2 values were compared for the CHX and Oil pulling groups, the difference was statistically significant (P=0).

CONCLUSION: The anti-plaque and anti-gingivitis activity of CHX was superior to Arimedadi Taila, though there was significant reduction observed in both groups. Overall, from baseline to II week and IV week, when the reduction in PlI, GI, BOP and GCF PGE_2 values were compared for the CHX and Oil pulling groups, the difference was statistically significant (P=0). In the Placebo group there was no anti-plaque and anti-gingivitis activity, which was in contrast to CHX and Oil pulling group. The present study results showed that PGE_2 levels in GCF were found to be more sensitive to changes in gingival inflammation.

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LIST OF ABBREVIATIONS

BOP	Bleeding on probing
CHX	Chlorhexidine
COX	Cyclooxygenase
ELISA	Enzyme linked immuno sorbent assay
GCF	Gingival crevicular fluid
GI	Gingival Index
Ig	Immunoglobulin
IL	Interleukin
MMP	Matrix metalloproteinase
NCTC	National Collection of Type Culture
OP	Oil pulling
PD	Probing depth
PDL	Periodontal ligament
PG	Prostaglandin
PL	Placebo
PlI	Plaque Index
PMN	Polymorphonuclear neutrophils
TNF	Tumor necrosis factor
HPLC	High performance liquid chromatography
HLA	Human leukocyte antigen
RIA	Radioimmunoassay
SRP	Scaling and rootplaning
QHI	Quigley-Hein plaque index
TxB	Thromboxane B

INTRODUCTION

In humans, dental biofilms accumulate on a daily basis. The basic research during the last century has clearly established the role of dental plaque at the interfaces of tooth and gingiva as the main cause of gingival inflammation, which could lead eventually to periodontitis.

Gingivitis, the commonest of all periodontal diseases is the inflammation of gingiva; characterized by host tissue inflammation due to bacterial plaque accumulation. It may be characterized by the presence of any of the following clinical signs: redness and edema of the gingival tissue, bleeding upon provocation, changes in contour and consistency, presence of calculus and/or plaque, and no radiographic evidence of crestal bone loss.[1] The conventional methods of controlling periodontal disease involve the mechanical removal of plaque & calculus. Maintenance of effective plaque control is the cornerstone of any attempt to prevent and control periodontal disease. However the quality of self performed mechanical plaque control is not sufficiently effective in most individuals and should be improved.[2]

Chemotherapeutic agents and antimicrobial agents are a common adjunct to mechanical hygiene measures to facilitate the control of supragingival plaque and gingivitis and its use has been known for decades. Mouth rinses represent one form of attack on oral microbes and malodor. The first reference to mouth rinsing around 2700 B.C. is credited to Chinese medicine literature for treatment of diseases of the gums by rinsing with urine of a child. We have come a long way since then to the use of chlorhexidine (CHX) now for over three decades.[3]

The advent of chemotherapy, antiseptics and antimicrobial compounds eventually paved way for a variety of chemical formulations which possessed anti-plaque activity. CHX is an antimicrobial agent and it deservedly has a place in the dental armory used to treat or prevent periodontal disease, and has earned its eponym of the gold standard against which other antiplaque and gingivitis agents are measured.[4]

Oil pulling, in CAM (Complementary and Alternative Medicine), is a procedure that involves swishing oil in the mouth for oral and systemic health benefits. It has been used extensively as a traditional Indian folk remedy for many years to prevent decay, oral malodor, bleeding gums, dryness of throat, cracked lips and for strengthening teeth and gums.[5] Ayurvedic books like Sushruta Samhita, & Charaka Samhita have mentioned the importance of Arimedadi Taila in the management of dental diseases. Arimedadi Taila was proved to be effective in reducing periodontal problems like bad breath, bleeding gums and swollen gums along with sensitivity of teeth in more than 80% of patients.[6]

Periodontal diagnosis and treatment plan are based on the assessment of clinical parameters and radiographic findings. However, these clinical parameters are not sufficiently sensitive and specific to identify disease activity in individual sites or to predict future attachment loss. Hence attention is focused on the development of diagnostic tools that could screen and differentiate the active inflamed sites and predict future tissue destruction.[7]

Prostaglandin E_2 (PGE_2) is one of the most potent biochemical mediators of inflammation which plays an important role in the pathogenesis of periodontal diseases. PGE_2 levels in gingival crevicular fluid (GCF) can serve as an indicator of the status of ongoing disease activity.[8]

Despite the wealth of information published, there has been little data on the impact of plaque control measures on PGE_2 levels and the role of PGE_2 in the progression of gingivitis. Hence, this study was planned to comparatively evaluate the efficacy of Chlorhexidine rinses and oil pulling with Arimedadi Taila in preventing plaque formation and progression of gingivitis. This study was also planned to assess the changes in GCF PGE_2 levels during health and gingivitis, and to assess the impact of plaque control measures on PGE_2 levels.

AIMS & OBJECTIVES

Aim:

To assess the clinical and biochemical changes that take place after the use of arimedadi OP in plaque induced gingivitis.

Objectives :

- To evaluate the efficacy of Arimedadi Taila in controlling plaque induced gingivitis;
- To compare the efficacies of CHX and Arimedadi Taila in preventing plaque formation and progression of gingivitis;
- To evaluate the levels of PGE_2 and its role in plaque induced gingivitis;
- To assess the impact of plaque control measures on GCF PGE_2 levels.

REVIEW OF LITERATURE

GCF AND ITS ROLE IN PERIODONTAL HEALTH AND DISEASE:

Waerhaug J (1952)[15] gave an early account of the presence of GCF in the 'gingival pockets' and their possible role in disease and health.

While investigating the physiological properties of the gingival pocket, he administered India ink into healthy gingival sulci of young experimental dogs. After 1hour, he observed increased fluid transudation and emigration of leukocytes and within the next 48 hours this 'transudate fluid' had eliminated most of the ink particles from the sulci. It was suggested, a flushing action of fluid originating from the gingival sulcus, which could aid in the removal of particulate matter from the sulcular space.

Brill and Krasse (1958)[16] in his experimental study, passed filter paper strips into the gingival sulcus of dogs, who were injected with sodium fluorescein. He demonstrated that parenterally administered tracer material could be recovered on strips of filter paper. However, no dye was detected on other intraoral locations, such as the teeth, tongue, palate, oral mucosa, and even extra-oral mucous membrane sites. These findings suggested that the epithelial lining of the gingival sulcus was permeable to small molecular weight compounds and that the passage of tissue fluid into the sulcus acts as a possible defense mechanism.[16,17]

Edelberg J (1966)[18] investigated the histology of the vasculature underlying the sulcular and junctional epithelium. A suspension of carbon particles was injected intravenously into the dogs, which were later sacrified and their gingival tissue was examined. The authors found that carbon particles remain in the capillaries and venules concluding that the production of GCF is primarily related to an increase in permeability of the vessels underlying the junctional and sulcular epithelium.

Bang and Cimasoni (1971)[19] utilized the Folin phenol protein quantitation method (proposed by Lowry et al 1951) to detect the total protein concentration of GCF and serum in 20 patients. They found that there was no significant difference between the protein concentrations in serum and GCF and thus concluded that GCF was an inflammatory exudate that passes the crevicular barrier, as a result of increased capillary permeability.[19] GCF flow changes can be monitored to detect subclinical inflammation, followed by analysis of chemical and microbial constituents for differentiating active and quiescent lesions.[20]

Alfano et al (1976)[21], through their human study postulated that proteinaceous substances increase GCF flow initially by establishing an osmotic gradient (transudative) and later by initiating inflammatory changes (exudative changes). In this study, baseline GCF samples were taken for one min. from the midlabial surface of two similar teeth from 12 healthy men. In the control teeth, phosphate buffered saline was introduced into the gingival sulcus while on the experimental teeth, an identical volume of phosphate buffered saline, modified to contain 10 mg/ml of homologous human serum albumin, was introduced. The flow rate was 84% higher on the experimental site at 3 hours and more than 100% higher at 6 hours compared to baseline levels. A hypothesis was put forth that in healthy gingiva a small amount of subgingival plaque gives rise to limited quantities of macromolecule by-products which will be removed by being absorbed to the surface of desquamating epithelial cells or through phagocytosis. When more molecules are present, they diffuse intercellularly to the basement membrane which can be considered as a major limiting barrier. As the macromolecules accumulate at the basement membrane, an osmotic gradient is created and the GCF is produced. GCF flow changes can be monitored to detect subclinical inflammation, followed by analysis of chemical and microbial constituents for differentiating active and quiescent lesions.[21]

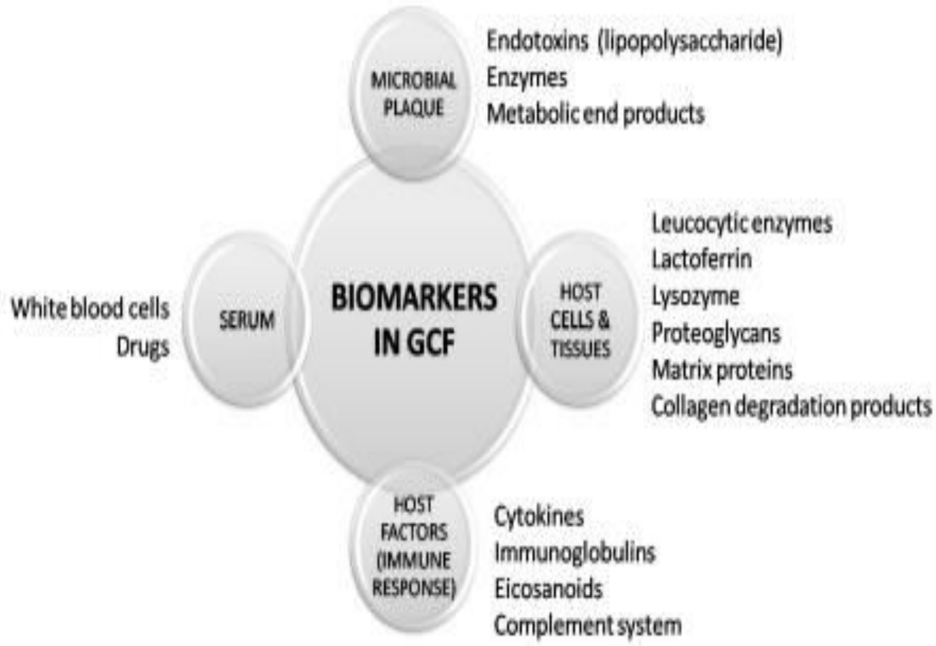
Pashley DH (1976)[22] developed a mathematical model based upon the starling factors governing fluid distribution across capillaries and the authors found that GCF production is modulated by capillary filtration and lymphatic uptake. From this, they concluded that when the production of fluid from capillaries is greater than the lymphatic uptake, it will accumulate as edema fluid or leave the area as GCF. GCF flow changes can be monitored to detect subclinical inflammation, followed by analysis of chemical and microbial constituents for differentiating active and quiescent lesions.

Curtis et al (1988)[23] estimated the total protein concentration by repetitive sampling for GCF over a 10 minute period. The mean concentration of the first sample was comparable to that of normal tissue fluids and lymph, irrespective of the state of inflammation of the sample site. However, during repeated sampling, the values rose to resemble serum protein levels, except at those sites with no clinically detectable inflammation. These findings are consistent with the hypothesis that, in health GCF represents the transudate of gingival tissue interstitial fluid but in the course of gingivitis and periodontitis, GCF is transformed into true inflammatory exudate.

Darany et al (1992)[24] measured the GCF flow rates and elastase activity in 56 patients at different levels of gingival inflammation. They found GCF flow rate to be more sensitive to early inflammatory changes leading to mild gingivitis, than GCF elastase activity.

Lamster and Novak (1992)[25] reviewed the literature pertaining to the contents of GCF and stated that GCF contains bio-markers which reflect the microbial, inflammatory and metabolic activities within the gingival sulcus/periodontal pocket. GCF flow changes can be monitored to detect subclinical inflammation, followed by analysis of chemical and microbial constituents for differentiating active and quiescent lesions.

Fig.1. Biomarkers in GCF



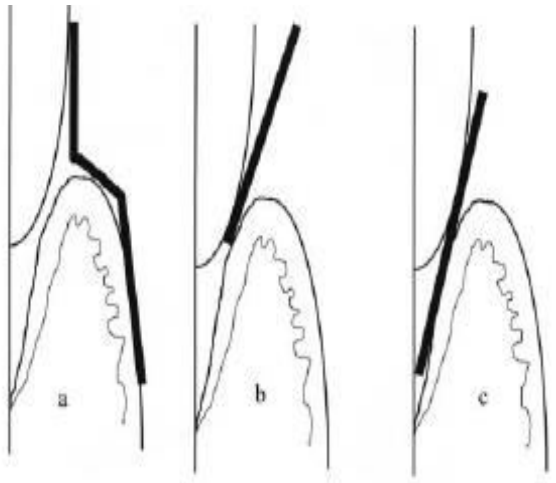
GCF COLLECTION METHODS

Brill and Krasse et al (1958)[16] recorded the occurrence of fluorescein in gingival pockets by means of filter paper strips. The strips were used in two different ways. In the intracrevicular method, the end was gently inserted into the pocket, whereas for the extracrevicular method the strips were placed on the vestibular surface of teeth, marginal and attached gingiva. **Weinstein et al (1967)** inserted preweighed twisted thread in to the gingival crevice around the tooth and determined the amount of fluid collected by weighing the sample.[26]

Griffiths GS (2003)[12] classified methods of GCF collection using filter strips, depending upon the placement, into intracrevicular technique, where the strips are placed at the entrance (Fig.2b) or base of the crevice/pocket (Fig.2c) and extracrevicular techniques, where the strips are overlaid on the gingival crevice region (Fig.2a).

The micropipette method of GCF collection is ideal as it provides an undiluted sample of 'native' GCF whose volume can be accurately assessed. There is less chances of any proteins or cells getting entangled during the collection process or retrieval, but it is difficult to collect an adequate volume of GCF in a short period, unless the sites are inflamed and contain large volumes of GCF.

Fig. 2. Different levels of placement of filter strips a) Extracrevicular method b) Intracrevicular method (superficial) c) Intracrevicular method (deep)



Micro-capillary tubes or micropipettes:

Sueda et al (1969)[27] in a quantitative analytic study, stated that GCF from the crevice migrates into the tube by capillary action and because the internal diameter is known, the volume of fluid collected can be accurately determined by measuring the distance to which the GCF has migrated**.**

Fig. 3. Collection of GCF using micropipettes

Gingival washings method:

Oppenheim FG (1970)[28] modified gingival washing technique, which involved the construction of a customized acrylic stent, isolating the gingival tissues from the rest of the mouth. The tissues were then irrigated for 15 min, with a saline solution, using a peristaltic pump and the diluted GCF was removed. The production of customized acrylic stents is complicated and technically demanding especially in the mandibular arch, because of the difficulties in producing a technically satisfactory appliance for the mandibular arch. It is also a disadvantage that GCF from individual sites cannot be analyzed. GCF flow changes can be monitored to detect subclinical inflammation, followed by analysis of chemical and microbial constituents for differentiating active and quiescent lesions.

Skapski & Lehner (1976)[29] introduced a crevicular washing method, in which the gingival crevice was perfused with an isotonic solution Hank's balanced salt solution. Instillation and re-aspiration of 10 ml of Hank's balanced salt solution at the interdental papilla was carried out. This process was repeated 12 times to allow thorough mixing of the transport solution and GCF. The fluid collected represented a dilution of crevicular fluid and contained both cells and soluble constituents such as plasma proteins. This technique could therefore be applied either to individual interdental units or to multiple units which were then pooled.

Bickel et al (1984)[30] inserted a periopaper strip (developed by Harco electronics Ltd) between the two jaws of periotron, which produces the flow of electric current and gives a digital read out on the screen. The volume of the fluid collected on the filter paper strips was measured. Translation of Periotron values to clinical conditions and GI with which they are associated are given below Tab.1[12]

Tab. 1. Translation of Periotron values to clinical conditions and GI

Periotron reading	Level of gingival inflammation	Gingival Index
0–20	healthy	0
21–40	mild	1
41–80	moderate	2
81–200	severe	3

Koregol et al (2011)[31] performed a clinico-biochemical study to estimate sodium, potassium and calcium concentrations of GCF in gingivitis and periodontitis to find the reliability of these ions as diagnostic markers and to analyze the relation of these ions to one another. This would indicate the stage of disease activity, which inturn helps in early diagnosis, prevention and treatment of periodontal diseases. The patients selected for the study included both sexes, aging from 18 to 55 years, divided into 2 groups: gingivitis (group I) and periodontitis (group II).

Using volumetric microcapillary pipette, 5 μl GCF was collected for quantitative analysis of sodium, potassium and calcium using flame photometry. The concentrations of sodium, potassium and calcium in GCF and their significant correlation with GI and pocket depth measurements, reflect the clinical status of gingival and periodontal tissues. In conclusion, estimation of these electrolytes may be used as potential diagnostic markers of active disease status in periodontal tissues and to predict the effective methods for prevention and treatment.

Guentsch et al (2011)[32] carried out a clinical study to compare three different sampling techniques to determine, whether one method yielded samples suitable for the reproducible and simultaneous determination of bacterial load, cytokines, neutrophil elastase and arginine-specific gingipains. GCF was sampled from four sites per patient (one sample per quadrant using two samples per method) in 36 patients with chronic periodontitis. One week later, the procedure was repeated with alternative methods.

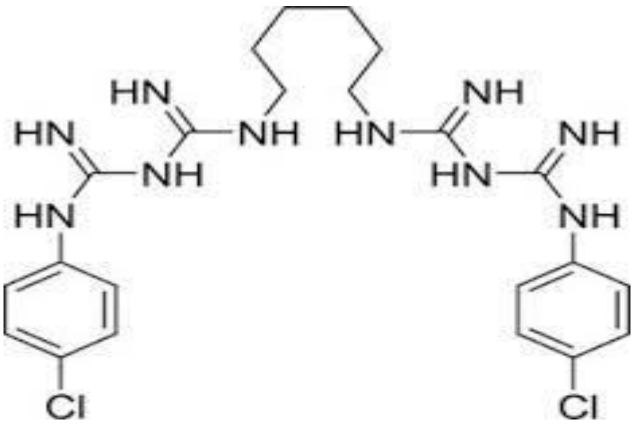
Variables determined were, loads of Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis, levels of IL-6 and -8, activity of neutrophil elastase and level of arginine-specific gingipais. The detected cytokine levels were higher using paper strips compared to paper points. Bacterial loads found in paper strips and paper points were similar. Arginine-specific gingipains were only detectable in high quantities by washing the periodontal pocket. The level of arginine-specific gingipains correlated with the load of Porphyromonas gingivalis.

In conclusion, they stated that use of paper strips was suitable for the simultaneous determination of microbial and immunologic parameters. Obtaining GCF by washing can be useful for special purposes. The gingipain concentration in periodontal pockets was directly determined to be $\leq 1.5 \mu M$. This value indicated that most of the substrates of these proteases identified until now can be easily degraded in Porphyromonas gingivalis-infected sites.

ROLE OF CHX MOUTHRINSES IN REDUCING GINGIVAL INFLAMMATION:

At physiological pH, CHX is a strong base and dicationic at P_H levels above 3.5 with two positive charges on either side of a hexamethylene bridge. It is chemically (1,6-di(4-chlorophenyl-diguanido) hexane.[33]

Fig. 4. Structure of CHX molecule



Mechanism of action: Antibacterial activity :

CHX has a wide spectrum of activity encompassing gram-positive and gram-negative bacteria, yeasts, dermatophytes and some lipophilic viruses. Its antimicrobial activity is of the membrane-active type, used to describe an antimicrobial agent that damages the inner (cytoplasmic) membrane. CHX shows different effects at different concentrations; at low concentrations the agent is bacteriostatic, whereas at higher concentrations the agent is rapidly bactericidal. The actual levels at which the bacteriostatic and bactericidal effects manifest themselves vary between bacterial species.[4]

The antibacterial mode of action of CHX is considered to be as follows: the bacterial cell is characteristically negatively charged and the cationic CHX molecule is rapidly attracted to the negatively charged bacterial cell surface, with specific and strong adsorption to phosphate-containing compounds. This alters the integrity of the bacterial cell membrane. CHX is attracted towards the inner cell membrane and binds to phospholipids in the inner membrane, leading to increased permeability of the inner membrane and leakage of low-molecular-weight components, such as potassium ions.[4]

At this bacteriostatic (sublethal) stage, the effects of CHX are reversible. Increasing the concentration of CHX causes progressively greater damage to the membrane, leakage of low-molecular weight cytoplasmic components falls, reflecting the coagulation and precipitation of the cytoplasm by the formation of phosphate complexes such as adenosine triphosphate and nucleic acids.[4]

Chawner and Gilbert (1989)[34] performed a comparative study of the bacteriostatic and bactericidal activities of alexidine and chlorhexidine. They suggested that there may either be specific binding sites for these molecules in the bacterial membrane or different intramolecular interactions of the two molecules at the membrane, the differences in the endgroup substitution between the biguanides affect their ability to produce lipid domains in the cell membrane.

Antiplaque effects:

Davies (1973)[35] considered that sucrose enhancement plaque formation may reduce the effects of CHX, such that the low “bacteriostatic” levels of CHX are no longer able to penetrate the cell wall of plaque bacteria grown in the presence of excess sucrose. At physiological pH, CHX is a large dicationic molecule, which has the ability to adsorb onto negatively charged surfaces, such as bacterial cell walls, where it exerts its bacteriostatic and bactericidal effects.

Addy M (1986),[36] in a review, ascribed CHX's antiplaque activity to its property of substantivity, which is the ability of an antimicrobial agent to persist within the oral cavity. He conducted an experimental study in a group of volunteers to compare the effects of a 0.2% CHX gluconate mouthrinse and a 0.035% Alexidine mouthrinse on plaque accumulation and salivary bacteria.

The subjects refrained from all forms of oral hygiene during the 10-day periods and rinsed twice a day with the mouthwash randomly allocated to the respective period. They found that after a single rinse with CHX, the saliva itself exhibits antibacterial activity for up to 5 hours.

CHX, also binds to the different surfaces within the mouth (teeth and mucosa) and also to the pellicle and saliva; for example, after a single rinse with CHX, the saliva itself exhibits antibacterial activity for up to 5 hours,[37] whereas persistence at the oral surfaces has been shown to suppress salivary bacterial counts for over 12 hours.[38]

Although CHX is able to bind to different anionically charged elements within the oral cavity, it also maintains its antibacterial activity for several hours.[37] Given that plaque formation occurs on the tooth surface, the binding of CHX to the pellicle-covered tooth surface was considered to be small compared with that involved in CHX-protein interactions at other oral surfaces.[35]

Jones CG (1997)[4] postulated that CHX, desorbed from the oral mucosa, might have three mechanisms of plaque inhibition: an influence on pellicle formation by blocking the acidic groups on the salivary glycoproteins, thus reducing the protein adsorption to the tooth surface; an influence on the adsorption of plaque onto the tooth surface by binding to the bacterial surface in sublethal amounts and an influence on the formation of plaque by precipitating the agglutination factors in saliva and displacing calcium from the plaque matrix.

For CHX to exert an antiplaque effect it must first be delivered successfully, in an active form, onto the tooth surface. On rinsing with CHX, there is an immediate reduction in the salivary bacterial counts. This reduction persists for several hours because of the substantivity of CHX, both within the saliva, and from mucosally desorbed CHX into the saliva.[4]

However, this is irrelevant to the inhibition of plaque by CHX. At the clean tooth surface a small amount of CHX binds to the pellicle and enamel, where it remains for several hours. This tooth surface-bound CHX can then interfere with the adherence of bacteria to the tooth surface through several mechanisms.[4]

Hjeljord et al (1973)[39] found that, in vitro reactions of CHX with soluble and precipitated proteins were reversible, with CHX, binding more strongly to precipitated proteins than to soluble proteins. The authors hypothesized that the CHX-protein interaction could involve a slight denaturation of the protein adsorbed to the teeth, and the slow reversibility of this reaction could partly explain the retention and slow release of CHX in the oral cavity. Similar effects seen with the more usual 10ml, 0.2% solution (20mg) can also be achieved with high volumes of low concentration solutions, as reported by **Lang et al (1981).**[40]

Jenkins et al (1988)[41] by topically applying 0.2% solutions of CHX only to the tooth surface, including by the use of sprays, reported the same level of plaque inhibition as rinsing with the full 20 mg dose & it showed that plaque growth on enamel inserts was inhibited equally well by 0.2% CHX applied topically or by rinsing. They observed enamel inserts under electron microscope and concluded that CHX achieved its antiplaque effect as a result of an immediate bactericidal action at the time of application, followed by a prolonged bacteriostatic action as a result of CHX adsorbing to the pellicle-coated enamel surface. This implied that the tooth surface-bound CHX was of greater importance in preventing plaque formation.

Jenkins et al (1994)[42] comparatively evaluated different doses of CHX for their anti-plaque activity against 0.1% triclosan. They found that even 0.01% CHX mouthrinse also had better anti-plaque efficacy which was superior to 0.1% triclosan rinse and considerable plaque inhibition was obtained with doses as low as 1-5mg twice daily. Their results revealed that anti-plaque effect of CHX was dose related

Efficacy of CHX mouthrinses:

Loe & Schiott (1970)[43] in an experimental human study, evaluated the effect of mouthrinses and topical CHX on the development of dental plaque and gingivitis, the authors concluded that rinsing with 10 ml of 0.2% w/v CHX gluconate mouthwash for 1 min, twice daily completely prevented the formation of plaque and gingivitis, provided the agent is administered in such a way that it reaches all tooth surfaces. Once rinsing with CHX was terminated, plaque formed again.

Cumming & Loe (1973)[44] showed that plaque control could also be achieved by lowering the concentration of CHX, whilst increasing the volume of the solution.

They concluded that the optimal dosage of CHX was 400 ml of a 0.025% to 0.05% solution as an oral irrigator and 50 ml volume of a 0.075% to 0.1% when used as a mouthwash.

Lang et al (1981)[40] compared several different regimens using mouthwashes with different concentrations and concluded that regular and daily use of 0.1% or 0.2% CHX mouthwash significantly reduced the development of plaque and gingivitis.

Brecx et al (1989)[45] showed that three daily rinses with 0.12% CHX gluconate matched the effect of optimally performed mechanical oral hygiene techniques. CHX has been studied in a number of controlled clinical trials. Tab. 2. In these studies, plaque reduction has ranged from 16-66% and gingivitis reduction has ranged from 24-80%.

Tab. 2. Clinical trials associated with the use of CHX mouthwash

Study	Trial length (months)	Concentration of CHX	Plaque reduction (%)	Gingivitis reduction (%)
Flotra et al (1972)[46]	4	0.2%	66	24
Lang et al (1982)[47]	6	0.1% 0.2%	16 19	67 80
Grossman et al (1989)[48]	6	0.12%	49	31
Loe et al (1976)[49]	24	0.2%	45	27

Toxicology, safety and side effects:

Flotra et al (1972)[46] in their 4-month study conducted to evaluate the effect of CHX mouthwash on 50 soldiers, summarized the side effects of CHX mouthrinse as follows -

1. Brown discoloration of the teeth, some restorative materials and of the dorsum of the tongue.
2. Taste perturbation, where the salt taste appears to be preferentially affected and leaves food and drinks with a bland taste.
3. Oral mucosal erosion which appears as an idiosyncratic reaction and concentration dependent. Dilution of the 0.2% formulation to 0.1%, but rinsing with the whole volume to maintain dose, usually alleviates the problem. Erosions are rarely seen with 0.12% rinse products used at 15 ml volume.
4. Unilateral or bilateral parotid swelling is an extremely rare occurrence and the reason for the same has not been identified. However, recent reports have attributed parotid gland swellings as a consequence of the rinsing action and not to the contents within mouthrinses.
5. Enhanced supragingival calculus formation which may be due to the precipitation of salivary proteins on to the tooth surface, thereby increasing pellicle thickness and/or precipitation of inorganic salts on to the pellicle layer. Bacterial resistance or evidence of super-infection by fungi, yeasts or viruses has not been reported with long-term oral use.

The phenomenon of surface adsorption appears fundamental to the antiplaque activity of the cationic antiseptics. Moreover, reaction with chromogenic material is relevant to the local side effect of staining.

Moran et al (1984)[50] performed a study inorder to determine, how such local reactions affect the antibacterial activity of some of these antiseptics. The minimum inhibitory concentration of commercial mouthrinses containing alexidine, cetyl pyridinium chloride, CHX gluconate and hexetidine against Oxford staphylococcus (National Collection of Type Culture - NCTC 6571) and Escherichia coli (NCTC 10418) was established by tube dilution.

The minimum inhibitory concentration of polymethylmethacrylate polymer on and of tea on Oxford staphylococcus was then measured. The zones of inhibition around acrylic blocks soaked in the respective antiseptics, with and without post exposure washings, were measured. The effects of tea on zone width of the antiseptic soaked blocks were recorded. The minimum inhibitory concentration values of alexidine, cetyl pyridinium chloride and CHX gluconate, but not hexetidine, were all increased by adding polymethylmethacrylate to cultures. Tea added to the culture increased the minimum inhibitory concentration values against E. coli for alexidine, CHX and hexetidine.

Zones of inhibition around antiseptic-treated blocks were reduced by washing and, in the case of hexetidine, completely abolished. Tea-soaking further reduced the zones of inhibition for alexidine and CHX, but not cetyl pyridinium chloride. It was concluded that surface adsorption and/or reaction with chromogenic material reduces the antibacterial activity of the cationic antiseptics. Hexetidine in the mouthrinse employed appeared to possess little or no adsorption potential to acrylic.

Baker et al (1987)[51] in an invitro study, assessed the antimicrobial effect of CHX and other compounds formed by replacement of side chains of CHX. The authors found that CHX acts by generalized perturbation of the bacterial membrane rather than in the antibiotic mode (lack of activity against specific bacterial enzymes or receptors), which decreased the chances for development of bacterial resistance.

Quirynen et al (2000)[52] conducted a clinical study was performed to examine the relative importance of the use of CHX in the one stage full-mouth disinfection protocol. Three groups of 12 patients each with advanced periodontitis were followed, both from a clinical and microbiological point of view, over a period of 8 months. The patients from the control group were scaled and root planed, quadrant per quadrant, at two-week intervals. The 2 other groups underwent a one stage full-mouth scaling and root planing (SRP) (all pockets within 24 hours) with full-mouth disinfection using CHX or without full-mouth scaling and root planing and the adjunctive use of CHX. At baseline and after 1, 2, 4 and 8 months, the following clinical parameters were recorded: PlI, GI, Probing depth (PD), BOP and clinical attachment level. Microbiological samples were taken from different intra- oral niches (tongue, mucosa, saliva and pooled samples from single- and multirooted teeth). The samples were cultured on selective and non-selective media in order to evaluate the number of colony-forming units/ml for the key-periodontopathogens.

All 3 treatment strategies resulted in significant improvements in all clinical parameters, but the full-mouth disinfection and full-mouth SRP patients reacted always significantly more favourably than the control group, with an additional PD reduction of 1.5 mm and an additional gain in attachment of 2 mm (for pockets >7 mm). Also from a microbiological point of view both the full mouth SRP and full mouth disinfection patients showed additional improvements when compared to the control group. The differences between full-mouth SRP and full-mouth disinfection patients were negligible. In conclusion, these findings suggest that the benefits of a ‘‘one-stage full-mouth disinfection’’ in the treatment of patients suffering from severe adult periodontitis.

Sekino et al (2003)[53] carried out a double-blind crossover clinical trial to study the effect of different CHX regimens on the number of bacteria in saliva, and on de novo plaque formation. Ten subjects with gingivitis, but no signs of destructive periodontitis, were recruited. Following a screening examination, the volunteers were given oral hygiene instruction, meticulous scaling and professional mechanical tooth cleaning. The professional mechanical tooth cleaning was repeated once every 3 days during a 2-week period to establish healthy gingival conditions. This includeed three phases. Each experimental phase comprised one preparatory period of 7 days and one plaque accumulation period (no oral hygiene measures) of 4 days.

During all preparatory periods, the volunteers (i) performed mechanical tooth cleaning using a toothbrush and dentifrice and (ii) were, in addition, given two sessions of professional mechanical tooth cleaning. The final professional mechanical tooth cleaning was delivered after bacterial sampling had been made on Day 0. In the preparatory period A, the participants continued the self performed plaque control regimen that employed only mechanical means. In the preparatory period B, the participants were in addition instructed to rinse and gargle, twice daily, with a 0.2% CHX mouthrinse. In the preparatory period C, in addition to the above, the participants were instructed to brush the dorsum of the tongue for 60 secs, twice daily, with a 1.0% CHX gel. Following each plaque accumulation period, there was a 10-day washout interval. The presence and amount of dental plaque Quigley-Hein plaque index (QHI) was scored after 1, 2 and 4 days of no oral hygiene.

Samples of saliva were obtained on Day 0 and after 1 and 2 days. The samples were placed on Brucella agar plates and incubated (anaerobically) for 5 days. The total number of colony-forming units was determined and used to estimate the density of bacteria in saliva. In period A, the mean QHI increased from 1.0 (Day 1) to 1.4 (Day 2) and 2.1 (Day 4). The corresponding scores for periods split B and C were 0.5, 0.8, 1.6 and 0.3, 0.8, 1.2, respectively. At all re-examination intervals more plaque formed during period A than during periods B and C. Further, during period C, less plaque formed than that during period B. Saliva samples from Day 0 in period A, contained a larger number of total viable counts than the baseline samples in periods B and C. There was no significant difference in total viable counts among the groups on Day 2. In conclusion, the daily use of CHX as an adjunct to mechanical tooth cleaning markedly reduced the number of microorganisms that could be detected in saliva.

Karpinski et al (2003)[54] performed a systematic review of pharmaco-biological activity and application of CHX and showed that it has strong biocidal activity against Gram-positive bacteria and weaker activity against Gram-negative bacteria. It is also active against yeasts, some dermatophytes and some lipophilic viruses. Numerous studies have confirmed the beneficial effects of CHX in reducing plaque accumulation, tooth caries, gingivitis, periodontitis and in alveolar osteitis. Unfortunately, CHX exhibits cytotoxic activity on human cells, can cause colorization of teeth and fillings and its activity depends on the pH of the environment and the presence of organic substances. In conclusion, CHX plays a valuable role in the dentistry and antisepsis. However, it can also cause side effects, limiting its application time.

Sharma et al (2009)[55] reported a case report of an immediate hypersensitivity reaction (urticaria) to CHX due to oral rinsing of CHX gluconate mouthwash (Rexidin mouthwash). The prevalence of contact urticaria and anaphylaxis due to CHX remains to be unknown. The adverse reaction was confirmed by skin prick test. After thorough oral examination of a 19-year-old female patient with the chief complaint of bleeding from the gums, she was advised oral prophylaxis and was prescribed CHX mouth rinse (Rexidin), twice daily for a period of three weeks.

Next day, patient presented with urticaria on her forehead and face, the front of the elbows and forearms, side and upper back region of the neck and on the lower abdomen. No oral changes were observed. On questioning, the patient reported using CHX mouthwash (Rexidin) formulation in a 1:1 concentration and rinsed with it for at least 1 min. On rinsing with it for the first time she noticed reddening on her forehead, face and side of the neck (after about 12 hour of using it). On waking up the next morning, she felt some burning sensation on the red spots, which she had noticed the previous night. Upon using the formulation again, after a couple of hours she observed marked redness on her upper back, neck region, lower abdomen, and on the front of the elbow and forearms. This was accompanied with irritation. History of any previous such allergic reactions were also recorded. To confirm that the allergic reaction was due to CHX, skin prick test was performed as follows: The inner forearm of the patient was cleaned with soap and water and was coded with a skin marker pen.

A drop of allergen (CHX) was then placed besides the mark. A small prick through the drop was made to the skin using a sterile prick lancet.

The excess allergen solution was dabbed off with a tissue. The skin under the drop of the CHX solution had become red and itchy within 30 min and this was surrounded by a white raised wheal. Hypersensitivity and other adverse reactions to CHX are rare, but its potential to cause anaphylactic shock is probably underestimated. In conclusion, CHX is the most effective and widely used antiplaque agent to date. Nevertheless, the present case report would remind the clinicians of an important potential risk of this widely used antiseptic and make them cautious before prescribing any CHX formulation as it may lead to local symptoms or even severe attacks.

Berchier et al (2010)[56] conducted a systematic review to evaluate the effects of 0.12% CHX mouthrinse compared with 0.2% on plaque and periodontal parameters. Plaque scores, parameters of periodontal inflammation and periodontal attachment loss were selected as primary outcome parameters. Screening of 409 titles and abstracts, eight eligible publications were identified. A meta-analysis of seven studies using the same PlI showed a significant difference between 0.2% and 0.12% CHX (p=0.008). Three studies that compared 0.12% and 0.2% CHX mouthrinse products showed no difference in the effect of gingivitis between the two concentrations.

In comparing 0.12% and 0.2% CHX, information concerning the effect on gingival inflammation was sparse and no studies could be found that compared the two CHX concentrations and evaluated the probing pocket depth and/or the periodontal attachment level. With respect to plaque inhibition, the results showed a small but significant difference in favour of the 0.2% CHX concentration. However, the clinical relevance of this difference is probably negligible.

Van Maanen-Schakel et al (2012)[57] carried out a systematic review, to search the literature concerning the inhibiting effect of an oxygenating agent on CHX induced tooth staining. Controlled / andomized controlled clinical trials conducted with healthy subjects > 16 years of age that compared the effects of CHX mouthrinse combined with an oxygenating agent with the effects of CHX alone were included. Combining an oxygenating agent with CHX mouthrinses led to a significant reduction in tooth staining, when compared with CHX alone. One of the included studies reported a side effect for one participant. Limited availability of data, and the included studies were methodologically and clinically heterogeneous, which affected the quality and interpretation of the evidence. In conclusion, there is moderate evidence that a combination of CHX and an oxygenating agent reduces tooth staining without interfering with plaque growth inhibition.

Pedrazzi et al (2014)[58] reviewed the importance of antimicrobial mouthrinse use as an auxiliary method in chemical peri-implant biofilm control. The active ingredients of the several available mouthrinses include bis-biguanide, essential oils, phenols, quaternary ammonium compounds, oxygenating compounds, chlorine derivatives, plant extracts, fluorides, antibiotics and antimicrobial agent combinations. It was concluded that there is strong clinical evidence that at least two mouthrinses have scientifically proven efficacy against different oral biofilms, i.e., CHX digluconate and essential oils; however, 0.12% CHX digluconate presents a number of unwanted side effects and should be prescribed with caution. Chemical agents seem beneficial in controlling peri-implant inflammation, although they require further investigation. A scientifically proven antiseptic, with significant short and long term efficacy and with no unwanted side effects, for the prevention and/or treatment of peri-implant disease was recommended.

Slot et al (2014)[59] performed a systematic review about the efficacy of CHX and tooth discoloration. Based on the existing scientific literature, the effect of CHX dentifrice/gel as compared to a regular or placebo dentifrice/gel is established in healthy adults on the primary outcome parameters of plaque and gingivitis scores. Tooth surface discoloration was evaluated as a side effect. Randomized controlled clinical trials, regarding self-performed brushing by adults without periodontitis, with a minimum duration of 4 weeks were included. Regarding plaque score reduction, the majority of the experiments using a CHX dentifrice provided a significant positive effect.

All studies assessing gingival bleeding as parameter for gingivitis observed a significant reduction in favour of CHX dentifrice over PL dentifrice. Tooth surface discoloration was more pronounced with CHX dentifrice. The combined data concerning parameters of interest for CHX gel compared with a placebo did not show a trend towards a beneficial effect on plaque and bleeding scores.

In conclusion, within the limitations of this analysis, it may be concluded that toothbrushing with a CHX gel does not provide conclusive evidence. Brushing with a CHX dentifrice can be effective with regard to the control of plaque and gingivitis. Tooth surface discoloration was observed as side effect, which can potentially have a negative impact on patients’ compliance.

Jose et al (2015)[60] carried out a randomized, examiner-blinded, parallel-group study to assess gingival bleeding following twice-daily use of 0.2% w/v CHX digluconate mouthrinse with and without alcohol (0.2% CHX-alcohol; 0.2% CHX-alcohol-free, respectively) and brushing with a standard fluoride toothpaste was compared to brushing alone. Three hundred and nineteen subjects with mild-to-moderate gingivitis were included. A prophylaxis was performed at baseline. Gingival Severity Index (GSI; primary objective), GI and PlI were assessed at baseline and after 6 weeks of treatment. Adverse events were recorded throughout the study.

Between treatment, differences at week VI, demonstrated significantly lower Gingival Severity Index for the 0.2% CHX-alcohol and 0.2% CHX-alcohol-free groups compared to brushing alone. There were also significant reductions in GI and PI for the 0.2% CHX-alcohol and 0.2% CHX-alcohol-free groups compared to brushing alone. The proportion of subjects reporting ≥1 treatment-related adverse events was 27.8% (0.2% CHX-alcohol), 24.8% (0.2% CHX-alcohol-free) and 3.7% (brushing alone).

In conclusion, CHX mouthrinse with or without alcohol, as an adjunct to brushing with regular fluoride toothpaste significantly reduces bleeding scores, plaque and gingival inflammation compared to brushing alone. Treatment-related adverse events are characteristic of those associated with the use of CHX and are similar for both mouthrinses.

Marrelli et al (2015)[61] carried out a clinical study to test the anti discoloration system (Curaden Healthcare) in comparison with the other mouthwashes without this system. Antiplaque activity showed by 3 of the most commercialized mouthwashes was tested, moreover, their ability in reducing the dental staining related to the oral use of CHX was also assessed.

The results demonstrated the clinical efficacy of the 3 mouthwashes, when compared with CHX. The anti discoloration system performed particularly well, with a clinical detection of dental staining significantly less than the others tested. This study demonstrated the clinical efficacy of anti discoloration system in the reduction of tooth staining, without a loss of antiplaque activity with respect to the competing mouthwashes containing CHX.

HERBAL MOUTHRINSES AND THEIR ROLE IN GINGIVAL AND PERIODONTAL DISEASES:

OP is an age-old process mentioned in Charaka Samhita and Sushrutha's Arthashastra. The process is called Kavala Gandoosha/kavala Graha in Ayurveda. This process is said to cure about 30 systemic diseases.[62] It has been used extensively as a traditional Indian folk remedy for many years to prevent decay, oral malodor, bleeding gums, and dryness of throat, and cracked lips and for strengthening teeth, gums, and jaws.[63] Panchakarma ayurvedic therapy which is a distinctive method of detoxifying the body offers great hope in future for development of strategies targeted towards prevention of gingival and periodontal diseases.[63]

Gandusha and Kavala Graha are two primary oral cleansing techniques; specialized therapy to treat as well as to prevent oral diseases. In Gandusha, the oral cavity is filled completely with liquid medicine, held for about 3-5 min or until nasal discharge or lacrimation occured and then released.

In Kavala Graha, the mouth is filled with a comfortable or three-quarters amount of fluid and is retained with the mouth closed for about 3 min. and then gargled. These oral cleansing techniques can benefit bad breath, dry face, dull senses, exhaustion, anorexia, loss of taste, impaired vision, sore throat and all kapha related imbalances.[5]

Other advantages of OP over commercially available chemical preparations include- it does not cause staining of teeth or allergic reactions and it does not leave behind a lingering taste.[14]

Abraham et al (2005)[65] performed a comparative study to evaluate the inhibitory activity of Triphala mouthrinse on Polymorphonuclear neutrophil-type MMP-9, in adult periodontitis patients.Tissue extracts from 10 periodontitis patients were treated with Triphala, doxycycline and kamillosan. The inhibition was analyzed by gelatin zymography, and the percentage of inhibition was determined by a gel documentation system. Comparison of type of mouthrinse used and its percentage inhibition of MMP activity is given in Tab. 2.

Tab. 3. Comparison of type of mouthrinsing & its inhibition of MMP-9 activity (%)

Type of mouthrinse	**Reduction of MMP-9 activity (%)**
Triphala	76.6%
Kamillosan (1,500 µg/ml)	46.36%
Doxycycline (300 µg/ml)	58.7%

Yun et al (2007)[66] investigated the inhibitory effect of (-)-epigallocatechin gallate, the main constituent of green tea polyphenols, on survival of osteoclasts, differentiated from Cells of the murine monocyte/macrophage cell line, Raw 264.7 cells. The effect of (-)-epigallocatechin gallate on osteoclast survival was examined by tartrate-resistant acid phosphatase staining in osteoclasts differentiated from RAW 264.7 cells.

Involvement of caspase in (-)-epigallocatechin gallate-mediated osteoclast apoptosis was evaluated by treatment with a general caspase inhibitor, Z-VAD-FMK, using a DNA-fragmentation assay.

Moreover, the effect of (-)-epigallocatechin gallate on the activation of caspase-3 was assessed by a colorimetric activity assay and western blotting. (-)-Epigallocatechin gallate significantly inhibited, in a dose-dependent manner, the survival of osteoclasts differentiated from RAW 264.7 cells and induced the apoptosis of osteoclasts.

Treatment with (-)-epigallocatechin gallate resulted in DNA fragmentation and induced the activation of caspase-3 in RAW 264.7 cell-derived osteoclasts. Additional treatment with Z-VAD-FMK suppressed these effects of (-)-epigallocatechin gallate.

In conclusion, it was suggested that (-)-epigallocatechin gallate might prevent alveolar bone resorption by inhibiting osteoclast survival through the caspase-mediated apoptosis. They suppressed the formation of osteoclasts by inhibiting the expression of matrix metalloproteinase-9 (MMP-9) in osteoblasts.

Asokan et al (2008)[67] evaluated the effect of sesame OP on the count of Streptococcus mutans in plaque and saliva of children and found that it provided significant reduction in Streptococcus mutans count.

Asokan et al (2008) [68] performed a comparative study to evaluate the effect of sesame OP and CHX rinses on plaque-induced gingivitis. They found statistically significant reduction of plaque and modified GI scores and a considerable reduction in the total colony count of aerobic microorganisms in both the groups.

Asokan et al (2008)[67] through a series of in vitro studies, assessed the possible anti-plaque action of OP therapy and they concluded that emulsification of sesame oil and a saponification process, evidenced by increased consumption of sodium hydroxide may play vital roles in the anti-plaque activity.

In a literature review of traditional therapies, the authors quoted references from 'Charaka Samhitha,' an ancient ayurvedic text about the use of a herbal preparation called Arimedadi Taila in the management of periodontal diseases.[69] The contents of a conventional Arimedadi Taila[6] have been listed in Tab. 4.

Tab. 4. Arimedadi Tailam Ingredients:

Paste prepared with 12 grams of each of fine powders of,

• Yashti – licorice- Glycyrhizaglabra • Trijatha- cinnamon, cardamom and cinnamomumtamala • Manjishta – rubiacordifolia • Gayatri – acacia catechu • Lodhra – symplocosracemosa • Katphala – myricanagi • Kshirivrikshatwak • Irimedatwak-acacia leucophloea/farnesiana • Musta – cyperusrotundus • Agaru – aquilariaagallocha • Shvetachandana – santalum album • Raktachandana–terocarpussantalinus • Karpoora,camphor– cinnamomumcamphora • Jati – myristicafragrans • Takkola – illiciumverum • Mamsi – nardostachysjatamansi • Dhataki – woodfordiafruticosa • Gairika – red ochre • Mrinala – cymbopogonjwarancusa • Mishi – anethumsowa • Vaidedi- piper longum • Padmakesara – nelumbonucifera • Kumkuma – crocussativus	• Laksha – lacciferlacca • Samanga, manjishta – rubiacordifolia • Brihati – solanumindicum • Bilvapatra – aeglemarmelos • Suradruma – cedrusdeodara • Shaileya – asphaltum • Sarala – pinusroxburghi • Sprikka – frlphiniumzalil • Palasha – buteamonosperma • Rajani – turmeric – curcuma longa • Daruharidra – berberisaristata • Priyangu – callicarpamacrophylla • Tejani – clematis triloba • Pradhakaleya – cosciniumfenestratum • Pushkara – inularaceomsa • Jaya – butilontheophrasti • Vyaghri – solanum xanthocarpum • Madana – randiadumetorum • Tila tila – sesame oil sesamuminidicum Decoction Arimeda- acacia leucophloea Nyagrodha-fiscusbengalensis Udumbara-fiscus racemosa Ashwattha-fiscus religiosa Plaksha-fiscus lacor

Likewise, the extracts of neem, guava and miswak have been used as dentifrices, chewing sticks, gels and mouthwashes[69] and mouth washes prepared from extracts of neem, guava, miswak, pomegranate, propolis, tulsi, craneberry and grapes[70] have also been shown to improve clinical parameters in patients with gingival diseases. However, researches pertaining to the biochemical, microbial and molecular effects of herbal products are still lacking and further studies are needed in order to better understand the potential benefit of these products.

Hosokawa et al (2010)[71] conducted an in vitro study about the catechin and found that it suppressed IL-6, C-C chemokine ligand 20 and CXC chemokine ligand 10 production in stimulated gingival fibroblasts. In an another in vitro study, **Tamura et al (2011)**[72] showed that catechins present in green tea derivatives can inhibit the growth of actinomyces, many periodontopathogens and oral candida species. This inhibition was hypothesized to be related to the generation of hydrogen peroxide, as the inhibitory effect was suppressed in the presence of catalase enzymes.

Dani et al (2015)[73] performed a clinico-microbiological study in forty patients with plaque induced gingivitis. The patients were treated with SRP with ultrasonic instrumentation, following this randomly, twenty patients performed OP (sesame oil) procedure for 14 days, another twenty patients used CHX mouthwash for 14 days. At baseline all subjects were instructed to wash their mouth with physiological saline (0.85% NaCl).This saline was collected in a sterile container and was serially diluted and plated in Mueller Hinton Agar plates.

The plates were incubated aerobically at 37˚C for 48hrs. After this incubation period the number of colonies present in 1ml of the saline was calculated by the formulae:

Number of bacteria/ml = Number of colonies dilution x Amount plated

OP with sesame oil, showed a greater reduction in the PlI scores by 51.6%, GI scores by 53.37% and total colony counts of aerobic microorganisms by 44.5% at 14 days, as opposed to 47.61%, 44.58% and 41.3% for the same in CHX group.

Studies on comparison of efficacy of CHX and Herbal mouth rinse:

Singh et al (2001)[5] conducted a single blind parallel randomized controlled trial in 48 volunteers involving four groups. The subjects were refrained from all oral hygiene measures for 4 days, but rinsed instead twice daily with 10 ml of a herbal mouthwash, essential oil, CHX or a PL solution. PlI and plaque area was assessed on day 4. The herbal mouthwash and essential oil showed a significant inhibition of plaque regrowth compared to PL ($P<0.001$), but the lowest values of PI and plaque area were obtained with CHX.

Statistically significant difference in plaque parameters was observed when CHX was compared to herbal mouthwash and essential oil, and herbal mouthwash to essential oil rinse. In conclusion, the new herbal mouthrinse had a promising plaque inhibitory potential, but it is not as efficacious as CHX in preventing plaque regrowth.

Bajaj et al (2011)[74] conducted a study in a total of 1431 students in the age group 8–12 years, belonging to classes fourth to seventh. The knowledge, attitude and practice of the subjects was determined using a questionnaire. The students were divided into three groups namely, Group I (n = 457) using Triphala mouthwash (0.6%), Group II (n = 440) using CHX mouthwash (0.1%) and Group III (n = 412) using distilled water. The assessment was carried out on the basis of plaque scores, gingival scores, and the microbiological analysis (Streptococcus and lactobacilli counts).

Both the Group I and Group II showed progressive decrease in plaque scores from baseline to the end of 9 months; however, for Group III increase in plaque scores from the baseline to the end of 9 months was noted. Both Group I and Group II showed similar effect on gingival health. There was inhibitory effect on microbial counts except Lactobacillus where Triphala had shown better results than CHX. It was concluded that there was no significant difference between the Triphala and the CHX mouthwash.

Shetty et al (2013)[75] conducted a study in 40 systemically healthy, chronic gingivitis subjects between the age groups of 18-25 yrs. The subjects received oral prophylaxis as part of phase 1 therapy after which they were asked to refrain from oral hygiene measures, and then randomly divided into 2 groups of 20 patients each, one prescribed CHX and the other, herbal mouthrinse (hiora) twice daily for 2 min. for four days. On day 5, subgingival plaque samples were collected for culture.

Clinical periodontal parameters viz PlI, GI and oral hygiene index were assessed on day 0 and day 5 and analyzed statistically. There were no statistically significant differences between the two groups with regard to the clinical parameters and colony counts of the bacteria. However, the CHX group showed statistically suggestive significance with respect to inhibition of Streptococcus mutans ($p<0.1$) and moderate significance with respect to inhibition of Aggregatibacter actinomycetemcomitans ($p<0.05$).

In conclusion, herbal mouth rinses may be as effective as CHX as chemical anti-plaque agents with fewer side effects. However, alternative study designs using larger sample sizes and longer duration are needed to further reiterate its benefits.

Naiktari et al (2014)[76] conducted a double-blind, randomized, multicenter clinical trial in 120 hospitalized patients showing clinical signs of gingival inflammation who were equally divided into three groups. Patients in group A were advised to rinse their mouths with 10 mL of distilled water, group B with 0.2% CHX, and group C with Triphala mouthwash for 1 min twice daily for two weeks.

The PlI and the GI were recorded on the first and the fifteenth day. There was no significant difference when the efficacy of Triphala was compared with 0.2% CHX. However, a statistically significant difference was observed in PI and GI when both group B and group C were compared with group A and also within groups B and C, with no statistically significant difference between groups B and C after 15 days ($P<0.05$). When group B was compared to group C, there was no statistically significant difference ($P>0.05$).

In conclusion, the Triphala mouthwash is an effective antiplaque agent like 0.2% CHX in reducing plaque accumulation and gingival inflammation. It is also cost effective, easily available, and well tolerable with no reported side effects.

Lakade et al (2014)[77] carried out a randomized controlled double-blind study to compare the antimicrobial efficacy of 0.2% CHX mouth rinse and mouth rinse containing 0.03% triclosan, 0.05% sodium fluoride, and 5% xylitol in reducing the

Streptococcus mutans count in plaque. Thirty healthy children aged 8-10 years with dmft (decay component) of three or four were selected.

They were divided randomly into two groups: The control or CHX group and the study group or combination mouth rinse. Both the groups practiced rinsing with respective mouth wash for 1 min. for 15 days, twice daily. The plaque samples were collected and after incubation Mutans streptococcus count was estimated on the strips from the Dentocult SM kit and evaluated using manufacture's chart. Statistically significant reduction in the Mutans streptococci count in the plaque was seen in the control and study group from baseline level. But when both the groups were compared, the antimicrobial effect of CHX was more.

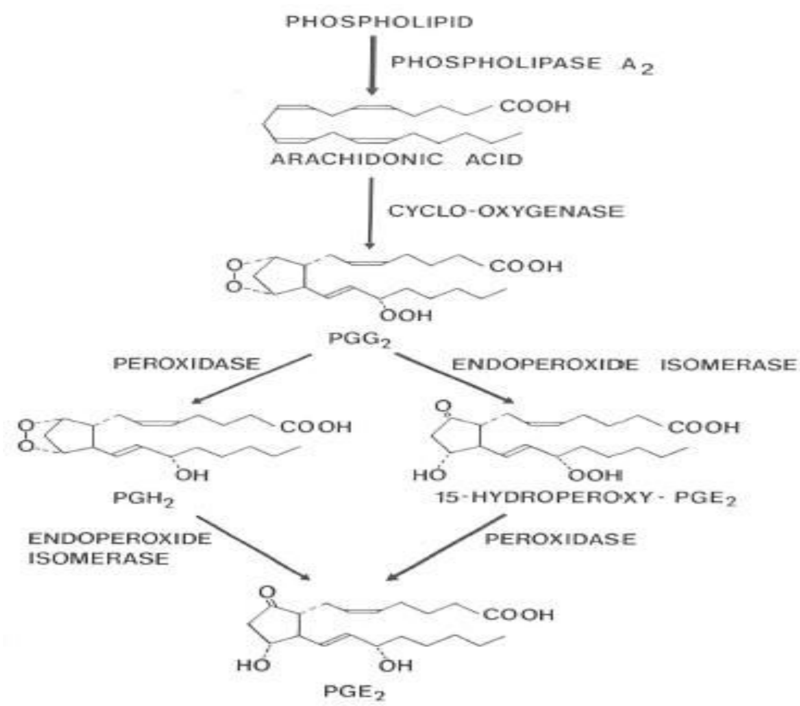
Gupta et al (2015)[78] conducted a double-blind randomized control trial among ninety undergraduate volunteer students. The students were randomly allocated into three study groups: 1) Terminalia chebula mouthwash, 2) CHX, 3) distilled water.

Assessment was carried out according to plaque score and gingival score. Results showed that Terminalia chebula mouthrinse is as effective as CHX in reducing dental plaque and gingival Inflammation. The results demonstrated a significant reduction of gingival bleeding and plaque indices in both groups over a period of 15 and 30 days as compared to the placebo. The authors concluded that Terminalia chebula extract mouthrinse can be used as an alternative to CHX mouthrinse as it has similar properties without the side-effects of the latter.

PGE AND ITS ROLE:

PGs are small-molecule derivatives of arachidonic acid (AA), produced by COXs (COX; constitutively active COX1 and inducible COX2 that convert AA into PGH_2, and PGE synthases, with a relatively minor contribution of the isoprostane pathway. Local levels of PGE_2 are regulated by the local balance between the COX2-driven synthesis and 15-hydroxyPGE dehydrogenase (15-PGDH)-mediated degradation of PGE_2. PGE_2 is one of the most abundant PGs produced in the body. It can be produced by all cell types of the body, with epithelia, fibroblasts, and infiltrating inflammatory cells representing the major sources of PGE_2 in the course of an immune response.

Fig. 5. Pathways of PGE_2 synthesis from arachidonic acid[79]



Receptors for PGE_2 :

The receptors for PGE_2 (EP1–EP4) are present on multiple cell types, reflecting the ubiquitous functions of PGE_2, which span nociception and other aspects of neuronal signaling, hematopoiesis, regulation of blood flow, renal filtration and blood pressure, regulation of mucosal integrity, vascular permeability, and smooth muscle function.[80]

EP4 and to a lesser extent EP2 receptors were involved in the inhibition of TNF-a production. PGE_2 suppresses production of chemokines including IL-8, monocyte chemoattractant protein-1, and macrophage inflammatory protein-1 via EP4 receptors in human macrophages stimulated with lipopolysaccharide.[81,82]

Role in inflammation:

In inflammation, PGE_2 is of particular interest because it is involved in all processes leading to the classic signs of inflammation. Redness and edema result from increased blood flow into the inflamed tissue through PGE_2 - mediated augmentation of arterial dilatation and increased microvascular permeability.[83] These effects are enhanced by synergism with other inflammatory mediators.[84,85]

It also possess anti-inflammatory properties, the development and regression of inflammation probably depends partly upon relative concentrations of pro- and anti-inflammatory PG activity and possibly also on a feedback inhibition by $PG_{S.}$[79]

Role in immunity:

PGE_2 supports acute local inflammation and phagocyte-mediated immunity at the site of pathogen entry, but it has a specialized role in controlling the potentially harmful activation of type 1 (cytotoxic) immunity, especially at later stages of immune responses. Dysregulated PGE_2 synthesis or degradation has been associated with a wide range of pathological conditions.[81] Although this ability of PGE_2 to limit type 1 immunity is crucial for the host self-preservation, it is counterproductive during infections with intracellular organisms, which both depend on enhanced PGE_2 production and/or on reduced degradation of PGE_2 for the establishment of immunosuppression and disease progression.

Although the therapeutic antagonism with the PGE_2 system has traditionally focused on the inhibition of PGE_2 production using non selective or COX2-selective blockers, the newly available agonists and antagonists of the individual PGE_2 receptors, allow for new therapeutic approaches to control the PGE_2-mediated immunopathology. Additionally, amplification of PGE_2 production and responsiveness to this factor and antagonizing its rate of decay may be used to treat autoimmune phenomena.[80]

Offenbacher et al (1984)[86] demonstrated that the concentration of PGE_2 in GCF correlates with the concentration of PGE_2 in the adjacent tissues and this was related to the existing attachment loss and it was approximately three times higher in GCF samples from patients with juvenile periodontitis compared to those with adult periodontitis. Subsequently, **Offenbacher et al (1986)**[87] studied patients with adult periodontitis to determine the longitudinal relationship between the concentration of PGE_2 in GCF and clinical attachment loss.

While increasing levels are seen from health to gingivitis to periodontitis,[88] healthy sites in patients with periodontitis experience also have higher levels of PGE, although levels are lower in maintenance patients than in patients with ongoing periodontitis reported by **Ebersole et al (1993).**[89] Thus individual site levels of PGE are difficult to reconcile with the clinical appearance of the site in isolation.

Offenbacher et al (1993)[90] found that the amount of PGE_2 released from stimulated cells of the monocyte/macrophage lineage (MØ) may have a genetic component. The association between MØ inflammatory mediator responses and Human leukocyte antigen (HLA) - haplotypes has profound implications in chronic inflammatory and autoimmune states. Patients having a high inflammatory response to LPS belonged to the HLA-DR4+ haplotype and the hyporesponsive subjects had HLA-DR2+ haplotype. This hyperresponsiveness due to HLA-DR4+ which has been found to be prevalent in diseases such as Rhematoid arthritis, Lupus Erythematosis and Diabetes mellitus could be the cause for susceptibility to severe and rapidly progressive forms of periodontitis.

PGE_2 is an inflammatory mediator and is found to be elevated in inflamed gingival tissue and GCF proportional to the severity of periodontal disease.[91,92] This was confirmed in a model of experimental gingivitis, where GCF PGE_2 levels increased at 4 weeks following cessation of oral hygiene procedures.[93] It was also suggested that what-ever the patient's phenotype (i.e., disease resistant or disease susceptible), there is a natural tendency for PGE_2 levels to gradually increase over time.[94]

Downie et al (1974)[95] conducted a clinical study to measure the levels of PGE in the human endometrium throughout the normal menstrual cycle, which were measured by bioassay. PGE_2 was found to be low during the proliferative phase of the cycle (10-25 ng/100 mg tissue). It remained lower during the luteal phase and was highest at menstruation (52ng / 100mg tissue). There is a cyclic variation in the levels of PGE in the endometrium. The physiological significance of these changes remains to be elucidated.

Mendieta et al (1984)[96] conducted a human study in 13 samples of inflamed human gingival tissue and 6 samples of normal human gingival tissue incubated with [l4C] arachidonate, PG metabolites were separated by thin-layer chromatography and identified by comparison with co-chromatographed standards. Inflamed gingival tissue synthesized, significantly larger amounts of 6-keto-PGF, thromboxane B2, PGD_2 and PGA_2 compared to normal tissue. Some unidentified metabolites, possibly lipoxygenase products, were detected in significantly larger amounts in inflamed than in normal tissue.

Ohm et al (1984)[92] conducted a clinical study in 50 gingival biopsies taken from 50 participants (aged 22 to 56 years), undergoing periodontal surgery. All patients were subjected to a presurgical treatment including oral hygiene instructions, repeated plaque control, and removal of dental calculus. None of the patients was undergoing medical treatment.

The classification into gingivitis and periodontitis was made by the aid of the Periodontal Index of Russell (1956). The index has a scale of values which increases from 0 to 8 with increasing prevalence and severity of disease. Values of 1 and 2 comprise the "gingivitis-group" and values from 6 to 8 comprise the "periodontitis-group."

Gingivitis was diagnosed by inflamed gingival with formation of supraalveolar pockets exceeding 2 mm without any reduction of the height of the alveolar bone crest. Surgical treatment of gingivitis was carried out by gingivectomy with an external bevel incision. Periodontitis was treated by flap surgery starting with a reversed bevel incision, so that the removed tissue was taken from the pocket and the gingival margin. Normal human gingival tissue was obtained during operative removal of impacted third molars. Clinical criteria for healthy gingival tissue were the absence of anomalous redness, no BOP, and a pocket depth not exceeding 2 mm distal of the second molar. Special attention was given to the fact that the third molar has had no connection to the oral cavity, so that there were no places for bacterial plaque to hide under a hood of inflamed mucosa behind the second molar. All biopsies were immediately frozen at -20°C in 0.9% sodium chloride solution.

The tissue samples of wet weight from 48 to 488 mg were homogenized and extracted twice with 6 ml of diethyl ether and once with 6 ml of ethyl acetate. Recovery values were about 95% after extraction.

The organic phase was evaporated to dryness under a stream of nitrogen at room temperature and the residue was redissolved in 200/µl of ethanol. Reversed phase High performance liquid chromatography (HPLC) was performed on a µ Bondapak "fatty acid analysis" column with the solvent system water:acetonitrile 74:26 v/v, pH 3.1, running at a flow-rate of 0.6 ml/min. The recovery values of the chromatographic step varied between 82 and 95%.

Quantitative analysis of each fraction was performed by radioimmunoassay (RIA) using commercially available antibodies. PGEs considered were shown to increase during advancing periodontal destruction. The most quantitatively significant endoperoxide metabolites at every stage of inflammation were TxB_2 and 6-keto-$PGF_{1\alpha}$, followed by PGE_2, 13,14Dihydro,15-Keto-PGE_2, PGE_1, $PGF_{1\alpha}$, $PGF_{2\alpha}$, and 13,14Dihydro,15-Keto -$PGF_{2\alpha}$ in decreasing order.

Alexander et al (1996)[97] carried out a clinical study to monitor IgGs, IL-lß, and PGE_2 in GCF of 18 adult patients, as they progressed through periodontal treatment toward maintenance therapy. Sites were selected from the most severely affected sextant as determined by PD at initial examination. GCF was collected on four occasions: initial examination; 4 weeks after completion of initial therapy (oral hygiene counseling, and SRP); 3 months after completion of surgery; and 7 to 9 months later at a maintenance visit. Significant improvements were observed for all the clinical variables measured: PD, attachment level, and BOP.

However, significant reductions for the GCF components only occurred in the concentrations of IL-1ß and PGE_2, but were not evident until the maintenance sampling. Surprisingly, GCF:serum ratios of IgG subclasses did not change significantly over the course of the investigation. The robustness of the levels of these components may be due to inflammation associated with the healing process, or to a further plaque induced response. [96]

Noguchi et al (1999)[98] carried out an experimental study to investigate the involvement of COX-1 and COX-2 in PGE, production by periodontal ligament (PDL) cells stimulated with a proinflammatory cytokine, IL -1α (IL -1 α), and to examine the regulation of PGE_2 production by cell-cell interaction of human gingival keratinocytes and PDL cells. It was found that IL-1α-stimulated PDL cells produced PGE_2 in a time-dependent manner.

Indomethacin, a non-selective COX-1/COX-2 inhibitor, and NS-398, a selective COX-2 inhibitor, completely inhibited PGE_2 production by the IL-1α - stimulated cells. COX-2 mRNA was detected after IL-1α stimulation, although it was not detected in unstimulated cells. There was no difference in expression of COX-1 mRNA between unstimulated cells and IL-lα stimulated cells. Expression of COX-2 protein in IL-1α -stimulated cells was increased, compared with that in unstimulated cells, whereas COX-1 protein expression was almost the same in both the cells.

Treatment of IL-1α -stimulated PDL cells with dexamethasone, known to inhibit COX-2 expression, prevented PGE_2 production and COX-2 mRNA expression. Addition of the culture media of human gingival keratinocytes to PDL cells increased PGE_2 production.

The PGE_2 production was depressed by treatment of the cells with IL-1 receptor antagonist and anti- IL-1α antibody, not with anti-IL-1β antibody. The PGE_2 production was also inhibited by treatment with NS-398 and dexamethasone. In conclusion, PDL cells stimulated with IL-1 α produce PGE_2 through de novo synthesis of COX-2 and that the cell interaction of gingival keratinocytes and PDL cells controls COX-2 expression and PGE_2 Production via IL-1 α or a IL-1 α-like factor.

Morton et al (2001)[99] conducted a research study in 32 gingival biopsies, obtained and processed histologically using hematoxylin and eosin, to determine the degree of inflammation. Of these biopsies, 7 with low and 7 with high histological levels of inflammation were further processed immunohistochemically in order to assess the levels of COX-2 expression in situ. To explore some potential mechanisms of COX-2 upregulation, gingival connective tissue primary cell cultures were established and challenged with periodontal bacteria or proinflammatory cytokines in vitro.

The levels of COX-2 expression were analyzed by western blot of cell lysates. COX-2 activity was assessed by quantifying PGE_2 levels in culture supernatants by competitive ELISA. The authors conclude that COX-2 expression is significantly upregulated in inflamed periodontal tissues. Both inflammatory cytokines such as IL-1β and bacterial constituents may be responsible for the enhanced COX-2 expression and PGE_2 synthesis in vivo.[100]

Rausch-Fan et al (2005)[100] performed a clinical study performed to investigate the influence of glycine on PGE_2 production. Human gingival fibroblasts were cultured in the presence of various concentrations of glycine and/or IL-1β, TNF-α, and IL-10 and their influence on PGE_2 production was measured. The expression of COX was analyzed by Western blot and immunocytochemistry.

The PGE_2 production by IL-1β-stimulated human gingival fibroblasts was significantly upregulated by glycine. The effect of glycine on IL- 1β-induced cell proliferation and PGE_2 production was concentration- dependent, reached a peak at 3 mM, and declined slowly at higher doses.

The synthesis of PGE_2 by human gingival fibroblasts cultured in the absence of glycine was significantly inhibited by IL-10 and partially induced in cells cultured with glycine. Glycine had no effect on TNF-α-induced PGE_2 production. The IL-1β-driven PGE_2 synthesis was blocked by indomethacin, a COX-1/COX-2 inhibitor, and by COX-2 inhibitor NS-398.

The expression of COX-2 protein was slightly induced by glycine, more evidently by IL-1β, and mostly enhanced by combined IL-1β with glycine. Since PGE_2 is a potent stimulator of bone resorption, and production of PGE_2 and COX-2 protein is augmented by glycine, our results strongly suggest that glycine may be involved in the pathogenesis of periodontitis.

Siegel et al (2007)[101] conducted a clinical study in 14 periodontally healthy subjects, who were divided into young (18–30 years, n=77) and elderly (46–77 years, n=7). A gingival biopsy was taken at baseline. After experimental gingivitis, clinical examination was repeated and a sec. biopsy was taken. The expression of COX-1, COX-2, COX3 and microsomal PGE synthase-1 was analyzed by means of immunohistochemistry. In both healthy age groups, COX-1 and microsomal PGE synthase-1 were expressed in epithelial cells, endothelial cells and fibroblast-like connective tissue cells.

COX-1 was found in Langerhans' cells of the epithelium. COX-2 expression was observed in cells exhibiting the morphology of epithelial mitosis cells, and the expression of COX-2 in periodontally healthy elderly subjects was significantly lower ($p \leq 0.05$). Following experimental gingivitis, COX-1 and microsomal PGE synthase-1 expression did not change.

However, the expression of COX-2 was significantly increased in both age groups ($p \leq 0.05$). COX-3 was not detected in any group investigated. In conclusion, COX-1 and microsomal PGE synthase-I were found to be expressed constitutively in gingival tissue, and expression was unaffected by age or inflammation states. In contrast, the expression of COX-2 was weaker in elderly subjects. In the course of experimental gingivitis, COX-2 was induced in both age groups.

Becerik et al (2010)[102] conducted a study in 25 gingivitis patients and 25 periodontally healthy subjects having regular menstrual cycles were included and observed at menstrual phase (1 to 2 days of), ovulation phase (12 to 14 days), and premenstrual phases (22 to 24 days). GCF and saliva samples were collected and clinical parameters including PlI and BOP were recorded at each menstrual phase.

Salivary estrogen and progesterone levels were analyzed to determine exact menstrual cycle days. GCF levels of IL-6, PGE_2, tissue plasminogen activator and plasminogen activator inhibitor were measured by enzyme-linked immunosorbent assay. The percentages of sites with BOP were significantly higher in menstrual phase (60.85-18.36) and ovulation phase (58.92-25.04) than in the premenstrual (40.12-20.10) phase in the gingivitis group ($P < 0.001$; repeated measures analysis of variance), whereas it was similar for all phases in the healthy group ($P > 0.05$; repeated measures analysis of variance). GCF levels of IL-6 were significantly elevated in gingivitis patients compared to healthy subjects in all phases (P=0.004, P=0.041, and P=0.046 for menstrual phase, ovulation phase, and premenstrual phase, respectively; Mann-Whitney U test).

GCF levels of IL-6, PGE_2, tissue plasminogen activator and plasminogen activator inhibitor were unchanged in different menstrual phases in both groups ($P >0.05$; Friedman test). In conclusion, the present study suggests that changes in the sex steroid hormones during menstrual cycles might have a limited effect on the inflammatory status of gingiva, but GCF cytokine levels were not affected.

Lai et al (2010)[103] in an in vitro study investigated the effects of areca nut extract on the production of COX-2 and the inflammatory mediator PGE_2 produced by PMNs. The possible effects of Areca nut extract on the production of COX-2 were examined using Western blotting analysis. The viability and production of PGE_2 of treated PMNs were determined using the propidium iodide staining method and the competition enzyme assay, respectively.

The possible pathways involved were also examined using the COX-2 inhibitor (NS398), the intracellular calcium chelator 1,2-bis(2-aminophenoxy) ethane-

N, N, N9, N9-tetraacetic acid tetrakis (acetoxymethyl ester) (BAPTA-AM), the p38 mitogen-activated protein kinase inhibitor (SB203580), and the extracellular signal-regulated protein kinase inhibitor (U0126).

Results showed that areca nut extract significantly induced the production of PGE_2 in a time- and concentration-dependent manner. This induction resulted from an increased expression of COX-2. Moreover, the application of BAPTA-AM, SB203580, and U0126 statistically significantly suppressed the induction of PGE_2. In conclusion, areca nut extract induced the production of PGE_2. The activation of the intracellular calcium concentrations, p38 MAPK, and ERK may be involved in the inducing effects of areca nut extract on PMNs. The findings suggest that areca nut chewing may induce an inflammatory response and affect the periodontal health of consumers.

Offenbacher S (2010)[104] performed a human study to characterize the changes in 33 biomarkers within the GCF during the 3-week induction and 4-week resolution of stent-induced, biofilm overgrowth mediated, experimental gingivitis in humans. Experimental gingivitis was induced in 25 subjects for 21 days followed by treatment with a sonic powered toothbrush for 28 days.

Clinical indices recordings and GCF collection were done weekly during induction and biweekly during resolution. Samples were analyzed using a bead-based multiplexing analysis for the simultaneous measurements of 33 biomarkers within each sample including cytokines, MMPs and adipokines.

PGE_2 was measured by ELISA. Statistical testing using general linear models with structured covariance matrices were performed to compare stent to contralateral (nonstent) changes in clinical signs and in biomarker levels over time. Gingivitis induction was associated with a significant 2.6-fold increase in IL 1-beta, a 3.1 fold increase in IL 1-alpha, and a significant decrease in multiple chemokines as well as MMP -1,- 3 and 13.

All changes in clinical signs and mediators rebounded to baseline in response to treatment in the resolution phase. In conclusion, stent-induced gingivitis is associated with marked, but reversible increases in IL1-alpha and 1-beta with suppression of multiple chemokines as well as selected mat MMPs.

Kumar et al (2013)[105] reported that the measurement of PGE_2 level in GCF has been shown to an indication of periodontal tissue destruction. The collection of GCF is a non-invasive procedure and hence developing PGE_2 chair side diagnostic kits to measure PGE_2 levels can not only estimate the nature of periodontal disease before treatment, but also after treatment. Changes in GCF PGE_2 levels following anti-plaque measures appears to have been less explored in the periodontal literature and need further exploration.

MATERIALS & METHODS

This study was conducted at the outpatient section of the Department of Periodontics, Sri Ramakrishna Dental College & Hospital, Coimbatore. Only patients who provided informed consent were recruited into the study.

STUDY DESIGN AND PATIENT SELECTION

This is a single center, triple blinded, randomized controlled clinical study. The subjects admitted to study had to satisfy the following criteria:

Inclusion criteria:

1. Age: More than 18 years and less than 45 years
2. Moderate to severe gingivitis (Loe & Silness GI scores between 1.1-3.0)

Exclusion criteria:

1. Pregnant and lactating women
2. Any systemic disease
3. Currently on any long term medications
4. Any periodontal treatment during the past 6 months
5. Smoking or other forms of use of tobacco

RANDOMIZATION & ALLOCATION CONCEALMENT

Twenty nine patients out of 36 out-patients (Drop outs–7 patients) with gingivitis (Fig. 6), who met the selection criteria and provided informed consent were randomized to 3 groups i.e. Group A mouthwash, Group B mouthwash, Group C mouthwash. Each patient is provided with any of the three types of mouthwashes i.e

CHX mouthwash or herbal oil mouthwash or Placebo mouthwash containing mint flavored distilled water. All the selected patients were given mouthwash which was blinded and assigned randomly in a sequential order. For example, the first patient, second patient and the third patient were given Group A mouthwash, Group B mouthwash and Group C mouthwash respectively. The groups to which the patients were randomized were written against their serial number in a small piece of paper and placed in a sealed envelope.

STUDY ETHICS AND SAFETY

The conduct of this clinical trial followed the principles in the Declaration of Helsinki (2008). Approval from the Institutional Review Board and Ethical Committee of Sri Ramakrishna Dental College and Hospital was obtained prior to implementation.

ARMAMENTARIUM

Diagnostic instruments

Mouth mirror, explorer, tweezer, periodontal probe, cotton rolls and pledgets. (Fig. 7)

Sample collection and storage

Micro-capillary pipette (1-5µl) (Sigma-Aldrich Co., St. Louis, USA)™, Sterile storage vials, Vial container and Ultralow temperature freezer (-70^{o}C) for storing GCF samples and Ultralow temperature freezer (-35^{o}C) for storing PGE_2 standard and conjugate. (Fig. 11).

ELISA analysis

PGE_2 ELISA kit (Fig.12), ELISA microplate reader (Fig. 18.), micro-pipettes of varying calibrations with tips. (Fig 12)

PGE_2 ELISA kit Contents:

1. Goat anti-Mouse IgG Microtiter Plate, (One Plate of 96 Wells)- is a plate using break-apart strips coated with goat antibody specific to mouse IgG.

2. PGE_2 EIA Conjugate (6 mL) is a blue solution of alkaline phosphatase conjugated with PGE_2

3. PGE_2 EIA Antibody (6 mL) is aA yellow solution of a monoclonal antibody to $PGE_{2.}$

4. Assay Buffer (30 mL) is a Tris buffered saline containing proteins and sodium azide as preservative.

5. Wash Buffer Concentrate (30 mL) is a Tris buffered saline containing detergents.

6. PGE_2 Standard, (0.5 mL) is a solution of 50,000 pg/mL PGE_2

7. pNpp Substrate (20 mL) is a solution of p-nitrophenyl phosphate in buffer which is ready to use.

8. Stop Solution (5 mL) is a solution of trisodium phosphate in water. It should be kept tightly capped as it is caustic in nature.

9. PGE_2 Assay Layout Sheets, one each was provided

10. Plate Sealer, one each was provided

Drug dispensing

Amber colored containers and measuring cup to dispense mouthwash. (Fig. 8).

GINGIVAL EXAMINATION

In every examination, the following clinical parameters were scored:

Gingival health was evaluated using

1. GI system (Loe and Silness 1963).[9]

2. Plaque was assessed using the criteria of the PlI system (Silness and Loe 1964).[10]

3. Gingival Bleeding was assessed using Modified Sulcular Bleeding Index (mSBI) (Mombelli et al, 1987).[11]

GCF COLLECTION

Gingival clinical examination was performed one day prior to GCF sample collection to avoid stimulation of the gingival tissues and blood contamination. The gingival status of the recruited patients was visually examined to assess possible GCF collection sites.

Next day, suitable sampling site which correlates with greatest gingival inflammation was selected, air dried and isolated with cotton rolls. The supragingival plaque was removed to avoid contamination and blocking of micro-capillary pipette. The tip of the color-coded, calibrated (1-3µl) volumetric micro-capillary pipette (Sigma-Aldrich Co., St. Louis, USA)™ was placed at the entrance of the gingival crevice. (Fig. 9.) The GCF samples which were entrapped with air bubbles or contaminated with blood or saliva were discarded and fresh samples were collected.[12]

Each micro-capillary pipette containing GCF was placed inside separate sterile tubes (Fig. 10) and stored at -70° C temperature[8] in an ultralow temperature freezer (HERA freeze -86°C Basic Upright Freezers, Thermo Fisher Scientific India Pvt. Ltd. Mumbai, India) in Department of Microbiology, Sri Ramakrishna Hospital, Coimbatore until further analysis. (Fig. 11)

MOUTHRINSES

The mouthrinses tested in the present trial were:

1. 0.2% CHX Digluconate (Hexidine mouthwash, ICPA Health Products LTD,Coimbatore)
2. Herbal mouthrinse (Arimedadi Taila) (Indian Medicines Pharmaceutical Corporation Ltd, (A Government of India Enterprise), Almora, Uttarakhand.
3. Placebo mouthrinse containing Mint flavoured distilled water (Prepared by adding 4 drops of mint flavouring agent to 300 ml of distilled water).

CHX, herbal oil mouthrinses and Mint flavored water mouthrinse were packed in individual amber colored containers of 300 ml each and was provided with a measuring cup.

ALLOCATION PROCEDURE

After collection of GCF samples, the patient was referred to the drug dispensing section of the Pharmacy of Sri Ramakrishna Dental College & Hospital, Coimbatore, where the sealed envelope corresponding to the patient's serial number was opened by a pharmacist who delivered the mouthwash mentioned in the envelope and also provided instructions pertaining to its use.

RINSING PROCEDURES

CHX group - rinsing with 10 ml of a mouthrinse containing 0.2% CHX Digluconate twice daily for 1 minute. The patients were instructed to rinse 30 minutes after brushing, and to avoid eating or rinsing the mouth with water for 30 minutes after the procedure.[13]

Herbal mouthrinse -.10 ml of Arimedadi Taila to be sipped, sucked, and swished between the teeth for 10-15 minutes (until the viscous liquid turns thin and milky white), twice daily after brushing. It should not be swallowed. It should be followed by washing with plenty of water.[14]

Mint flavored water group - rinsing with 10 ml of a mint flavored water mouthrinse twice daily for 1 minute. The patients were instructed to rinse 30 minutes after brushing, and to avoid eating , drinking or rinsing the mouth with water for 30 minutes after the procedure.

FOLLOW UP

The patients were instructed to report after two weeks, GCF sample collection procedures were performed and the patients were re-examined to observe any changes in the gingival clinical parameters that were measured at baseline. At second week they were given fresh mouthwashes after returning the empty bottles.

At the end of fourth week, similar GCF sample collection procedures were performed and the patients were re-examined to observe any changes in the gingival clinical parameters that were measured at second week.

ELISA ANALYSIS

The biochemical evaluations were done at the Department of Biochemistry, Bioline Laboratory, Coimbatore, Tamilnadu.

Assay Procedure:

On the day of ELISA analysis, GCF samples which were stored at -70° C were thawed and brought to room temperature. And all reagents should be brought to room temperature for at least 30 minutes prior to opening.

1. PGE_2 Standard preparation:

   50,000 pg/mL PGE_2 standard solution was allowed to warm to room temperature. Seven, 12 x 75 mm glass tubes were labelled #7 till #1. 1ml of standard diluent was pipetted (Assay Buffer or Tissue Culture Media) into tube #7. 500µl of standard diluent was pipetted into tubes #6 till #1. 50µl of diluent was removed and 50µl of the 50,000 pg/mL standard was added to tube #7. It was vortexed thoroughly. 500µl of solution from tube #7 was added to tube #6 and vortexed thoroughly. 500µl of solution from tube #6 was added to tube #5 and vortexed. This was continued for tubes #4 through #1. The concentration of PGE_2 in tubes #7 through #1 was 2,500, 1,250, 625, 313, 156, 78.1 and 39.1 pg/mL respectively. (Fig.14)

2. Wash buffer was prepared by diluting 5mL of the supplied concentrate with 95 mL of deionized water.

3. The Assay Layout Sheet was referred in order to determine the number of wells that were to be used .

4. From each micro-capillay pipette 1µl of the GCF sample was transferred to 99 µl of standard human assay buffer, which was provided with the ELISA kit by passing the plunger through the micro-capillary pipettes.(Fig. 15)

5. 100 µL of standards #1 through #7 was pipetted into the appropriate wells.

6. 100 µL of the samples were pipetted into the appropriate wells.

7. 50 µL of blue conjugate (Fig. 16.a) was pipetted into each well, except the Blank wells.

8. 50 µL of yellow antibody (Fig. 16.b) was pipetted into each well, except the Blank. Every well used was required to be green in color.

9. The plate was incubated at room temperature on a plate shaker for 2 hours at ~500 rpm. The plate was covered with the plate sealer provided..

10. The contents of the wells were emptied and manually washed by adding 400 µL of wash solution to every well. The wash was repeated 2 more times for a total of 3 washes.

11. After the final wash, the wells were emptied and aspirated, and the plate was tapped firmly on a lint free paper towel to remove any remaining wash buffer.

12. 200 µL of the pNpp substrate solution was added to every well and incubated at room temperature for 45 minutes without shaking

13. 50 µL of stop solution (Fig.16.c) was added to every well. This stopped the reaction and the plate was read immediately.

14. The absorbance was measured using a microplate reader (Model 680 microplate reader, Bio-Rad Laboratories Inc, USA) after the recommended incubation process. (Fig. 17). Standard curve was plotted by the reader. (Graph.1). Sample concentrations may be calculated off Net OD values using desired curve fitting. The plate reader was blanked against the Blank wells and the optical density was read at 405 nm, with correction at 590 nm. The minimal detectable dose of PGE_2 was 13.4 pg/mL.

DEPARTMENT OF PERIODONTICS

SRI RAMAKRISHNA DENTAL COLLEGE & HOSPITAL

FORM I – SCREENING PROFORMA

"A RANDOMIZED CLINICAL TRIAL TO EVALUATE THE EFFICACY OF ARIMEDADI OIL PULLING IN PLAQUE INDUCED GINGIVITIS AND ITS EFFECT ON METABOLIC MARKER IN GINGIVAL CREVICULAR FLUID".

1. Serial No. ____________________Code No.________________________

2. Name of the subject ____

3. Date of Birth | | | | Age (in years) | | |

4. Postal address____

____

Telephone No.____

5. CRITERIA OF INCLUSION:

	Yes	No
1. Age: More than 18 years and less than 45 years		
2. Moderate to severe gingivitis (Loe & Silness Gingival Index scores between1.1-3.0)		

6. CRITERIA OF EXCLUSION

	Yes	No
1. Pregnant and lactating women		
2. Any systemic disease		
3. Currently on any long term medications		
4. Any periodontal treatment during the past 6 months		
5. Smoking or other forms of use of tobacco		

7. Quantity of mouth rinse provided to the patient ______________________________

Dated ______ Signature of the Doctor ___________________________

FORM 1A-HISTORY PROFORMA

1. Educational status

Illiterate ☐ Educated ☐

If educated then mention level of education___________________________

2. Occupation____

3. Monthly family income (in INR) _____________________________

4. Chief complaint with duration (if any) in weeks.

	Absent	Present	Duration
1. Bleeding gums			
2. Bad breath			
3. Pain in gums			
4.Bleeding on brushing/eating only			
5. Itching of gums			
6. Swelling in gums			
7. Pus discharge from gums			

8. Any other complaint's (Specify) ________________________________

5. Personal history:

1. Diet: Veg ☐ Non –veg ☐ Mixed ☐

2. Brushing habit:____

3. Any other (specify)____

Date_______ Signature of the doctor____________________

DEPARTMENT OF PERIODONTICS

SRI RAMAKRISHNA DENTAL COLLEGE & HOSPITAL

FORM II – CLINICAL ASSESSMENT

"A RANDOMIZED CLINICAL TRIAL TO EVALUATE THE EFFICACY OF ARIMEDADI OIL PULLING IN PLAQUE INDUCED GINGIVITIS AND ITS EFFECT ON METABOLIC MARKER IN GINGIVAL CREVICULAR FLUID".

Baseline ☐

CLINICAL PARAMETERS ASSESSMENT:

17 16 15 14	13 12 11 21 22 23	24 25 26 27
47 46 45 44	43 42 41 31 32 33	34 35 36 37

COLOR

CONTOUR

CONSISTENCY

SURFACE TEXTURE

BLEEDING ON PROBING

CLINICAL INDICES:

GINGIVAL INDEX (LOE & SILNESS, 1963)

	17	16	15	14	13	12	11	21	22	23	24	25	26	27
B														
P														

	47	46	45	44	43	42	41	31	32	33	34	35	36	37
B														
L														

Calculation: $\dfrac{\text{Sum of score of each teeth}}{\text{Total number of teeth examined}} =$

Inference: ……………………...

Mild gingivitis: 0.1 - 1.0

Moderate gingivitis: 1.1 – 2.0

Severe gingivitis: 2.1 – 3.0

PLAQUE INDEX (SILNESS & LOE, 1964)

	17	16	15	14	13	12	11	21	22	23	24	25	26	27
B														
P														

	47	46	45	44	43	42	41	31	32	33	34	35	36	37
B														
L														

Calculation: $\dfrac{\text{Sum of score of each teeth}}{\text{Total number of teeth examined}} =$

Inference: ……………………..

Excellent: 0

Good: 0.1 - 1.0

Fair: 1.1 – 2.0

Poor: 2.1 – 3.0

BLEEDING INDEX: (MODIFIED SULCULAR BLEEDING INDEX, MOMBELLI ET AL, 1987)

17	16	15	14	13	12	11	21	22	23	24	25	26	27
47	46	45	44	43	42	41	31	32	33	34	35	36	37

Calculation: $\dfrac{\text{Sum of score of each teeth}}{\text{Total number of teeth examined}} =$

Date ____________ Signature of the Doctor ___________________________________

DEPARTMENT OF PERIODONTICS

SRI RAMAKRISHNA DENTAL COLLEGE & HOSPITAL

FORM II – CLINICAL ASSESSMENT

"A RANDOMIZED CLINICAL TRIAL TO EVALUATE THE EFFICACY OF ARIMEDADI OIL PULLING IN PLAQUE INDUCED GINGIVITIS AND ITS EFFECT ON METABOLIC MARKER IN GINGIVAL CREVICULAR FLUID".

II week ☐ **IV week** ☐

1. Serial No. ☐

2. Code No. of the subject____________________________________

3. Name of the subject ______________________________________

4. Date of birth ☐☐☐☐☐☐

Age (in years) ☐☐

5. Date of assessment_________

6. CLINICAL SYMPTOMS:

	Absent	Present
1. Bleeding gums		
2. Bad breath		
3. Pain in gums		
4. Bleeding on brushing or eating only		
5. Itching of gums		
6. Swelling in gums		
7. Pus discharge from gums		

8. Other complaints, if any (specify) ____

____

____

____

7.

CLINICAL PARAMETERS ASSESSMENT:

	17 16 15 14	13 12 11 21 22 23	24 25 26 27
	47 46 45 44	43 42 41 31 32 33	34 35 36 37
COLOR			
CONTOUR			
CONSISTENCY			
SURFACE TEXTURE			
BLEEDING ON PROBING			

8. Adverse reactions: Yes ☐ No ☐

9. Overall clinical assessment by the doctor:

Improved ☐ No change ☐ Deteriorated ☐

10. Overall impression of well being by the subject:

Complete cure ☐ Improved ☐ No change ☐ Deteriorated ☐

11. Status of the patient:

Continuing ☐

Drop out ☐

Reason____

____

CLINICAL INDICES:

GINGIVAL INDEX (LOE & SILNESS, 1963)

	17	16	15	14	13	12	11	21	22	23	24	25	26	27
B														
P														

	47	46	45	44	43	42	41	31	32	33	34	35	36	37
B														
L														

Calculation: $\frac{\text{Sum of score of each teeth}}{\text{Total number of teeth examined}}$ =

Inference: ……………………

Mild gingivitis: 0.1 - 1.0

Moderate gingivitis: 1.1 – 2.0

Severe gingivitis: 2.1 – 3.0

PLAQUE INDEX (SILNESS & LOE, 1964)

	17	16	15	14	13	12	11	21	22	23	24	25	26	27
B														
P														

	47	46	45	44	43	42	41	31	32	33	34	35	36	37
B														
L														

Calculation: $\dfrac{\text{Sum of score of each teeth}}{\text{Total number of teeth examined}} =$

Inference: ....

Excellent: 0

Good: 0.1 - 1.0

Fair: 1.1 – 2.0

Poor: 2.1 – 3.0

BLEEDING INDEX : (MODIFIED SULCULAR BLEEDING INDEX, MOMBELLI ET AL, 1987)

17	16	15	14	13	12	11	21	22	23	24	25	26	27
47	46	45	44	43	42	41	31	32	33	34	35	36	37

Calculation: $\dfrac{\text{Sum of score of each tooth}}{\text{Total number of teeth examined}} =$

Date _______

Signature of the Doctor___________________

DEPARTMENT OF PERIODONTICS

SRI RAMAKRISHNA DENTAL COLLEGE & HOSPITAL

PATIENT INFORMATION SHEET

"A randomized triple blind controlled clinical trial to evaluate the efficacy of arimedadi oil pulling in plaque induced gingivitis and its effect on metabolic marker in gingival crevicular fluid".

What is the study about?

The present study is aimed to evaluate the efficacy of Arimedadi Taila in the management of gum diseases. Arimedadi Taila is a medicated oil which contains ayurvedic medicaments.

What will you have to do?

As a participant of this study, you must rinse your mouth twice daily with 10 ml of the medicated oil or a regular allopathic mouthrinse or a placebo mouthrinse for a period of one month. The interval between subsequent visits during the treatment will be 2 week. At each visit you will be supplied with sufficient quantities of drug to last until your next visit (2 week). After completion of rinsing for 1 month, you will be required to visit the hospital for necessary check up.

Before you start treatment, during the first visit to the clinic, you will undergo a complete oral examination and there after on next day about 1 µl fluid that gets secreted in your gums will be collected. The same procedures will be repeated at II week and IV week.

DEPARTMENT OF PERIODONTICS

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"A randomized triple blind controlled clinical trial to evaluate the efficacy of arimedadi oil pulling in plaque induced gingivitis and its effect on metabolic marker in gingival crevicular fluid".

CONSENT FORM

CERTIFICATE BY INVESTIGATOR

I certify that I have disclosed all details about the study in the terms easily understood by the patient.

Date______________

Signature______________________

Name________________________

CONSENT BY SUBJECT

I have been informed to my satisfaction, by the attending physician, the purpose of the clinical trial and the nature of drug treatment and follow-up, including the laboratory investigations to be performed to monitor and safeguard my body functions.

I am also aware of my right to opt out of the trial at any time during the course of the trial without having to give the reasons for doing so. I, exercising my free power of choice hereby give my consent to be included as a subject in this clinical trial which involves the evaluation of Arimedadi Taila in plaque induced gingivitis.

Date________________ Signature or Thumb impression

Date________________ Signature of witness_____________

Name________________________

Relationship___________________

DEPARTMENT OF PERIODONTICS

SRI RAMAKRISHNA DENTAL COLLEGE & HOSPITAL

FORM 3 – LABORATORY INVESTIGATIONS

"A RANDOMIZED CLINICAL TRIAL TO EVALUATE THE EFFICACY OF ARIMEDADI OIL PULLING IN PLAQUE INDUCED GINGIVITIS AND ITS EFFECT ON METABOLIC MARKER IN GINGIVAL CREVICULAR FLUID".

1. Serial No.

2. Code No. of the subject________________________________

(to be filled later by the investigator)

3. Samples received on ______________________________

4. Date of assessment_________________________________

5. Lab investigations_________________________________

INFLAMMATORY BIOMARKER	QUANTITY	COMMENTS
PROSTAGLANDIN E_2		
GCF SAMPLE 1		
GCF SAMPLE 1		
GCF SAMPLE 1		

Date______________ Signature of the lab technician

STATISTICAL ANALYSIS :

The distribution of PGE_2 levels in GCF were examined to identify potential outliers. Changes in GI scores, PlI scores, Bleeding scores and GCF PGE_2 levels at baseline, II week and IV week following mouth rinsing was assessed using paired samples 't' test and the statistical significance was determined using two-sided p values ($p<0.05$). Comparison of CHX and OP group (P values) was assessed by using independent samples 't' test and $P <0.05$.was considered statistical significant.

FIGURES

Fig. 11. Ultra low temperature freezers that were used for storing samples at -70°C and -35°C

Fig. 12. ELISA kit

Fig. 13. Micro-pipettes used for transfering reagents and samples

Fig. 14. Addition of standard and assay buffer

Fig. 15. Addition of GCF sample

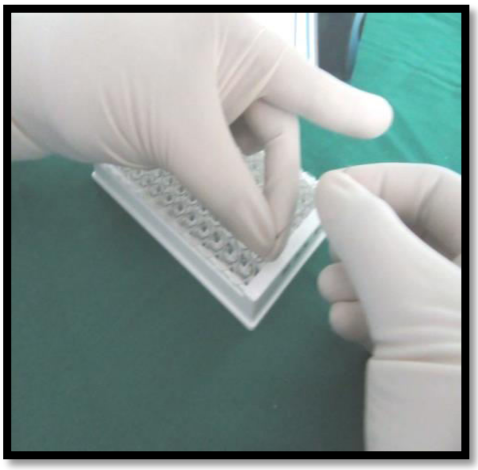


Fig. 16. Different steps during ELISA- following addition of

(a) Conjugate (b) Antibodies (c) Stop solution

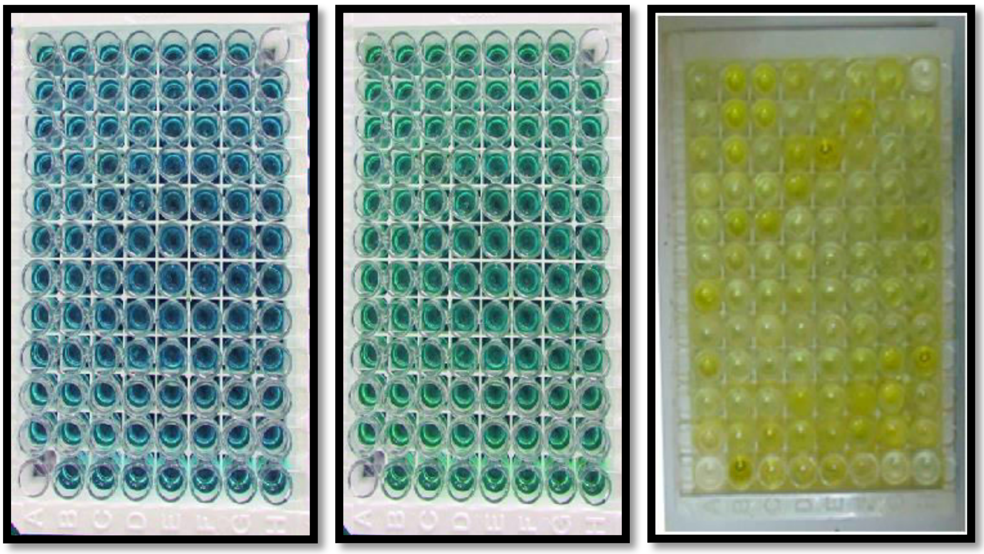
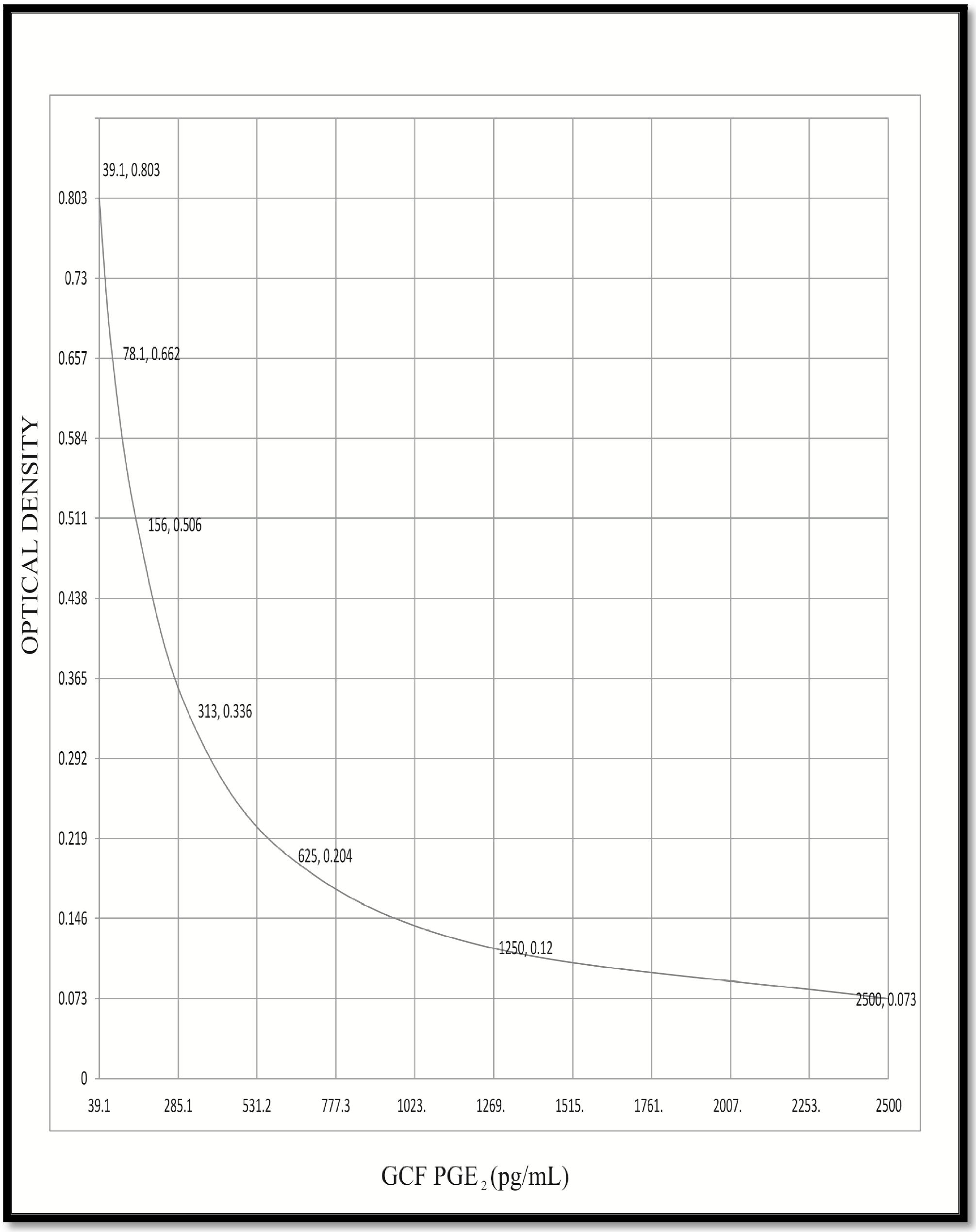


Fig .17. Microplate reader used for measuring absorbance

Graph 1: Standard graph



RESULTS

The efficacy of Arimedadi Taila on gingivitis patients has been studied by evaluating its effect on clinical and biochemical parameters and the observations are as follows -

1. BASELINE SAMPLE CHARACTERISTICS:

The mean age (± SD) of participants with gingivitis was 27.36 ± 6.76 years (18 – 45 yrs). Out of 29 patients number of males were 15 (48.28%) and females were 14 (58.72%). Sample collection was done at baseline, II week and IV week from 10 patients in Oil pulling group, 10 patients in CHX group and 9 patients in Placebo group. The data pertaining to the subject variables for all the 3 groups i.e., Age, sex and brushing frequency/day have been summarized in Tab. 5.

2. BASELINE AND POST-INTERVENTION (II WEEK AND IV WEEK) CLINICAL PARAMETERS AND PGE_2 LEVELS IN GINGIVITIS PATIENTS:

The inflammatory status of the gingiva around the tooth with greatest inflammation (from which the GCF sample was taken) was assessed using the Gingival Index (Loe & Silness, 1963). The overall Gingival Index (Loe & Silness, 1963), Plaque Index (Silness & Loe, 1964) and Modified Sulcular Bleeding Index (Mombelli et al, 1987) score have been summarized in Tab. 6, which also lists the PGE_2 levels in GCF of the corresponding patient, both at baseline, second week and fourth week.

3. COMPARISON OF BASELINE AND POST-INTERVENTION VALUES FOLLOWING MOUTHRINSING:

b. Changes in Plaque index (PlI) score post-intervention:

In the CHX group, PlI score at baseline, II week and IV week were 1.808, 1.504, and 1.163 respectively. Overall, the PlI score decreased by 16.79% from baseline (1.808) to II week (1.504), and 22.69% from II week (1.504) to IV week (1.163). This reduction was statistically significant (P=0). And also, when the PlI score was compared from baseline to IV week, there was a reduction of 35.67%, which was statistically also significant (P=0).

In the Oil pulling group, PlI score at baseline, II week and IV week were 1.910, 1.851 and 1.793 respectively. It decreased by 3.07% from baseline (1.910) to II week (1.851) and by 3.13% from II week (1.851) to IV week (1.793), and this reduction was statistically significant (P=0). Furthermore, when the PlI score was compared from baseline to IV week, there was a reduction of 6.11%, which was also statistically significant (P=0).

In the Placebo group, PlI score at baseline, II week and IV week were 1.996, 1.998 and 2.043 respectively. The PlI score increased by 0.10% from baseline (1.996) to II week (1.998), but this increase was not significant (P=0.719). However, when the PlI score at II week (1.998) and IV week (2.043) were compared, it further increased by 2.29% at IV week and this increase was statistically significant (P=0.012). And also, when the PlI score was compared from baseline to IV week, there was an increase of 2.39%, which was also statistically significant (P=0.025).

Overall, from baseline to IV week, when the Placebo, CHX and Oil pulling groups were compared, the percentage reduction in PlI score was highest in the CHX group (35.67%), when compared to the Oil pulling group (6.11%). In contrast, the Placebo group demonstrated an overall increase in PlI score (2.39%). The data related to the same has been depicted in Fig. 19 and Tab. 7.

Finally, from baseline to II week and IV week, when the reduction in PlI were compared for the CHX and Oil pulling groups, the difference was statistically significant (P=0). (Tab. 8.)

b. Changes in Gingival Index (GI) score post-intervention:

In the CHX group, GI score at baseline, II week and IV week were 1.727, 1.395, 1.187.respectively. Overall, the GI score decreased by 19.19% from baseline (1.727) to II week (1.395), and 14.94% from II week (1.395) to IV week (1.187). This reduction was statistically significant (P=0). And also, when the GI score was compared from baseline to IV week, there was a reduction of 31.26%, which was also statistically significant (P=0).

In the Oil pulling group, the GI score at baseline, II week and IV week were 1.808, 1.742 and 1.645.respectively The GI score decreased by 3.67% from baseline (1.808) to II week (1.742), and this reduction was statistically significant (P=0.004).

From II week (1.742) to IV week (1.645), the GI score further decreased by 5.56% and this decrease was statistically significant (P=0.001). And also, when the GI score was compared from baseline to IV week, there was a reduction of 9.03%, which was also statistically significant (P=0).

In the Placebo group, GI score at baseline, II week and IV week were was 1.936, 1.939 and 1.996 respectively. It increased by 0.15% from baseline (1.936) to II week (1.939), but this increase was not significant (P=0.674). However, when the GI score at II week (1.939) and IV week (1.996) were compared, it further increased by 2.96% at IV week and this increase was statistically significant (P=0.003). Furthermore, when the GI score was compared from baseline to IV week, there was an increase of 3.12%, which was also statistically significant (P=0.011).

Overall, from baseline to IV week, when the Placebo, CHX and Oil pulling groups were compared, the percentage reduction in GI score was highest in the CHX group (31.26%), when compared to the Oil pulling group (9.03%). In contrast, the Placebo group demonstrated an overall increase in GI score (3.12%). The data related to the same has been depicted in Fig. 20 and Tab. 7.

Finally, from baseline to II week and IV week, when the reduction in GI scores were compared for the CHX and Oil pulling groups, the difference was statistically significant (P=0). (Tab. 8.)

c. Percentage reduction in gingival bleeding post-intervention:

The data pertaining to the difference between the percentage of sites showing signs of bleeding on probing (BOP) at baseline and after intervention has been summarized in Fig. 21 and Tab. 7. In the CHX group, bleeding score at baseline, II week and IV week were 79.166%, 65.952% and 51.785% respectively. Overall, the bleeding sites decreased by 16.69% from baseline to II week, and by 21.48% from II week to IV week. This reduction was statistically significant (P=0). And also, when the bleeding score was compared from baseline to IV week, there was a reduction of 34.59%, which was also statistically significant (P=0).

In the Oil pulling group, bleeding score at baseline, II week and IV week were 78.214%, 75.952% and 73.571% respectively. It decreased by 2.89% from baseline to II week and by 3.13% from II week to IV week, this reduction was statistically significant (P=0). Furthermore, when the bleeding score was compared from baseline to IV week, there was a reduction of 5.94%, which was also statistically significant (P=0).

In the Placebo group, the bleeding score at baseline, II week and IV week were 73.280%, 73.809% and 76.290% respectively. It increased by 0.72% from baseline to II week, but this increase was not significant (P=0.104). However, when the bleeding score at II week and IV week were compared, it further increased by 3.36% at IV week and this increase was statistically significant (P=0.005). Furthermore, when the Bleeding score was compared from baseline to IV week, there was an increase of 4.11% which was also statistically significant (P=0.009).

Overall, from baseline to IV week, when the Placebo, CHX and Oil pulling groups were compared, the percentage reduction in bleeding score was highest in the CHX group (34.59%), when compared to the Oil pulling group (5.94%). In contrast, the Placebo group demonstrated an overall increase in bleeding score (4.11%).

Finally, from baseline to II week and IV week, when the reduction in BOP scores were compared for the CHX and Oil pulling groups, the difference was statistically significant (P=0). (Tab. 8.)

d. Changes in GCF PGE_2 levels post-intervention:

The changes in GCF PGE_2 levels (Pg/mL), from baseline to post-intervention have been summarized in Tab. 7 and Fig. 22. In the CHX group, crevicular PGE_2 levels at baseline, II week and IV week were 200.899 Pg/mL, 147.028 Pg/mL and 103.106 Pg/mL respectively. It decreased by 26.81% from baseline to II week and by 29.87% from II week to IV week. This reduction was statistically significant (P=0). Furthermore, when the PGE_2 levels were compared from baseline to IV week, there was a reduction of 48.68%, which was also statistically significant (P=0).

In the Oil pulling group, crevicular PGE_2 levels at baseline, II week and IV week were 194.929 Pg/mL, 182.677 Pg/mL and 173.286 Pg/mL respectively. It decreased by 6.29% from baseline to II week, and this decrease was statistically significant (P=0). From II week to IV week, crevicular PGE_2 levels decreased by 5.14% and this reduction was statistically significant (P=0.001). And also, When the PGE_2 levels were compared from baseline to IV week, there was a reduction of 11.10%, which was also statistically significant (P=0).

In the Placebo group, the crevicular PGE_2 levels at baseline, II week and IV week were 171.439 Pg/mL, 173.351 Pg/mL and 186.838 Pg/mL respectively. It increased by 1.11% from baseline to II week, but this increase was not significant (P=0.674). However, when the PGE_2 levels at II week and IV week were compared, it further increased by 7.78% at IV week and this increase was statistically significant (P=0.001). Furthermore, when the PGE_2 levels were compared from baseline to IV week, there was an increase of 8.98%, which was also statistically significant (P=0.003).

Overall, from baseline to IV week, when the Placebo, CHX and Oil pulling groups were compared, the percentage reduction in crevicular PGE_2 levels was highest in the CHX group (48.68%), when compared to the Oil pulling group (11.10%). In contrast, the Placebo group demonstrated an overall increase in GCF PGE_2 levels (8.98%).

Finally, from baseline to II week and IV week, when the reduction in GCF PGE2 levels were compared for the CHX and Oil pulling groups, the difference was statistically significant (P=0). (Tab. 8.)

Tab. 5. Baseline sample characteristics

Parameter	No. of Patients	No. of Males	No. of Females	Brushing frequency/day		Age (years)
				Once	Twice	Mean±S.D
Total	29	15	14	19	10	27.36± 6.76
Oil pulling	10	5	5	6	4	25.9 ± 4.09
CHX	10	5	5	7	3	30.67 ± 6.95
Placebo	9	5	4	6	3	25.67 ± 8.29

Tab. 6. Baseline and post-intervention clinical parameters and GCF PGE_2 levels

Patient	Group	Tooth with greatest GI scores	Period	Pll scores	GI scores	BOP scores	GCF PGE_2 levels (pg/mL)
1	OP	2.25	Baseline	2.035	2.071	85.714	198.009
			II week	1.964	2.000	82.142	186.481
			IV week	1.892	1.892	80.952	174.721
2	CHX	2.5	Baseline	1.892	2.446	85.714	270.619
			II week	1.535	1.750	72.619	194.418
			IV week	1.178	1.642	59.523	140.407
3	PL	2.5	Baseline	2.607	2.205	88.095	235.210
			II week	2.616	2.223	89.285	240.120
			IV week	2.669	2.294	92.857	266.762
4	OP	2.75	Baseline	1.562	2.526	95.238	318.241
			II week	1.508	2.428	94.047	299.145
			IV week	1.500	2.285	90.476	280.051
5	CHX	2.5	Baseline	1.607	2.044	79.761	188.000
			II week	1.333	1.705	67.857	137.642
			IV week	0.991	1.616	55.952	98.004
6	PL	2.25	Baseline	1.616	1.910	69.047	157.582
			II week	1.616	1.919	70.238	161.280
			IV week	1.625	2.035	75.900	174.721
7	PL	2.5	Baseline	2.241	2.232	80.952	211.683
			II week	2.250	2.250	80.952	213.842
			IV week	2.330	2.303	84.523	235.210
8	OP	2.0	Baseline	1.830	1.919	92.857	306.015
			II week	1.794	1.892	89.285	287.642
			IV week	1.759	1.767	86.904	268.280
9	CHX	1.5	Baseline	1.437	1.133	58.333	133.972
			II week	1.205	0.946	48.809	103.001
			IV week	0.839	0.776	39.285	68.640
10	OP	1.25	Baseline	1.910	1.151	76.190	163.620
			II week	1.848	1.125	75.000	153.802
			IV week	1.732	1.044	73.809	147.841

Patient	Group	GI of tooth with greatest inflammation	Period	PlI scores	GI scores	BOP scores	GCF PGE_2 levels (pg/mL)
11	CHX	1.5	Baseline	1.741	1.352	83.333	257.581
			II week	1.428	1.153	67.857	183.963
			IV week	1.017	0.954	52.381	128.163
12	PL	2	Baseline	2.017	1.955	67.857	144.180
			II week	2.026	1.973	67.857	147.843
			IV week	2.098	2.000	70.238	161.282
13	OP	2.5	Baseline	1.562	2.303	90.476	254.002
			II week	1.526	2.196	86.904	235.210
			IV week	1.473	2.098	83.333	227.448
14	CHX	2.5	Baseline	2.116	2.285	96.428	360.009
			II week	1.785	1.946	79.761	262.801
			IV week	1.455	1.607	63.095	180.600
15	PL	2.5	Baseline	2.267	2.053	79.761	198.009
			II week	2.276	2.071	80.952	203.047
			IV week	2.330	2.142	84.523	217.811
16	OP	2.5	Baseline	2.375	2.187	89.285	273.060
			II week	2.304	2.089	86.904	252.945
			IV week	2.232	1.982	84.523	245.754
17	CHX	2.25	Baseline	2.017	1.276	91.666	176.003
			II week	1.723	1.098	75.000	125.006
			IV week	1.437	0.901	57.142	87.481
18	PL	1.5	Baseline	1.017	1.205	59.523	96.720
			II week	1.035	1.205	60.714	96.720
			IV week	1.044	1.276	63.095	104.641
19	OP	1.5	Baseline	1.285	1.196	57.142	84.241
			II week	1.241	1.125	55.952	78.343
			IV week	1.196	1.089	53.571	74.763
20	CHX	2.5	Baseline	2.446	1.821	78.571	163.620
			II week	2.044	1.482	65.476	119.882
			IV week	1.642	1.151	51.190	84.241

patient	Group	GI of tooth with greatest inflammation	Period	Pll scores	GI scores	BOP scores	GCF PGE_2 levels (pg/mL)
21	PL	2.25	Baseline	2.437	2.107	69.047	150.971
			II week	2.446	2.107	70.238	153.182
			IV week	2.571	2.214	71.428	171.008
22	OP	1.75	Baseline	2.017	1.357	63.095	101.002
			II week	1.964	1.285	60.714	94.997
			IV week	1.901	1.232	57.142	90.299
23	CHX	2.5	Baseline	2.394	2.116	63.095	115.002
			II week	2.035	1.776	51.190	87.482
			IV week	1.687	1.419	39.285	59.283
24	PL	2	Baseline	2.107	1.723	71.428	168.000
			II week	2.071	1.678	70.238	165.611
			IV week	2.080	1.687	69.047	163.623
25	OP	2	Baseline	2.258	1.732	67.857	131.222
			II week	2.160	1.687	65.476	123.541
			IV week	2.062	1.571	63.095	117.007
26	CHX	1.5	Baseline	1.160	1.250	73.809	144.182
			II week	0.937	1.063	63.095	108.244
			IV week	0.687	0.812	51.190	76.003
27	PL	2.25	Baseline	1.651	2.035	73.809	180.600
			II week	1.642	2.026	73.809	178.510
			IV week	1.642	2.017	75.000	186.481
28	OP	2	Baseline	2.267	1.642	64.285	119.882
			II week	2.205	1.589	63.095	114.661
			IV week	2.187	1.491	61.904	106.693
29	CHX	2	Baseline	1.267	1.544	80.950	200.002
			II week	1.017	1.035	67.857	147.841
			IV week	0.696	0.991	48.809	108.242

Tab. 7. Comparison of baseline and post-intervention values following mouthrinsing

Comparison of Baseline to II Week scores

	PL				CHX				OP			
	Baseline	II week	% Increase	P value	Baseline	II week	% Decrease	P value	Baseline	II week	% Decrease	P value
PlI	1.996	1.998	0.10%	0.719	1.808	1.504	16.79%	0.000	1.910	1.851	3.07%	0.000
GI	1.936	1.939	0.15%	0.674	1.727	1.395	19.19%	0.000	1.808	1.742	3.67%	0.004
BOP	73.280	73.809	0.72%	0.104	79.166	65.952	16.69%	0.000	78.214	75.952	2.89%	0.000
GCF	171.439	173.351	1.11%	0.076	200.899	147.028	26.81%	0.000	194.929	182.677	6.29%	0.000

Comparison of II week to IV week scores

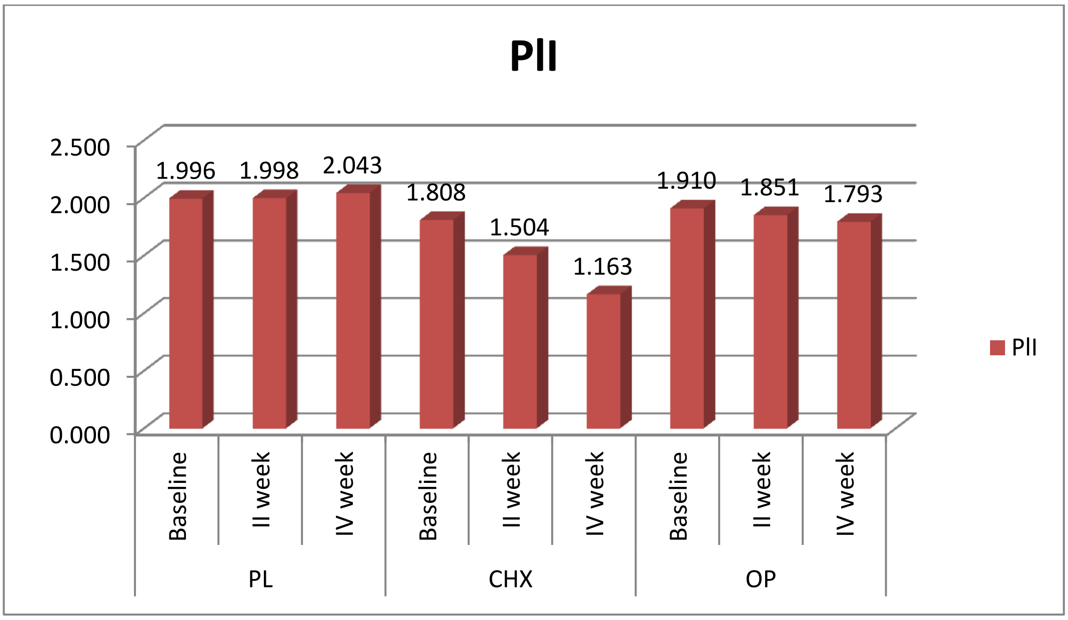
	PL				CHX				OP			
	II week	IV week	% Increase	P value	II week	IV week	% Decrease	P value	II week	IV week	% Decrease	P value
PlI	1.998	2.043	2.29%	0.012	1.504	1.163	22.69%	0.000	1.851	1.793	3.13%	0.000
GI	1.939	1.996	2.96%	0.003	1.395	1.187	14.94%	0.000	1.742	1.645	5.56%	0.001
BOP	73.809	76.290	3.36%	0.005	65.952	51.785	21.48%	0.000	75.952	73.571	3.13%	0.000
GCF	173.351	186.838	7.78%	0.001	147.028	103.106	29.87%	0.000	182.677	173.286	5.14%	0.001

Comparison of Baseline to IV Week scores

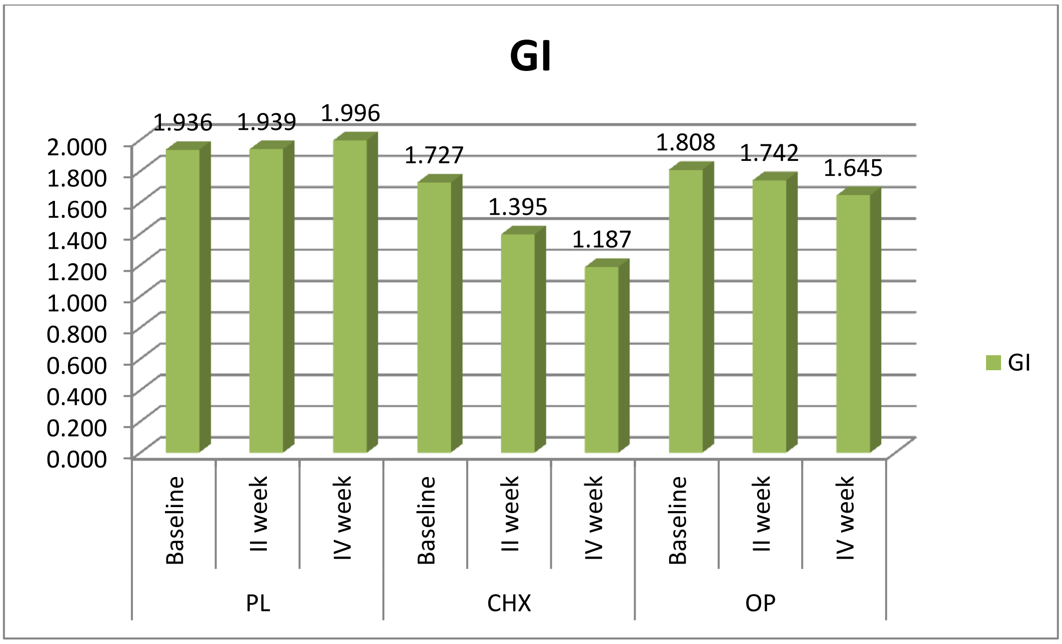
	PL				CHX				OP			
	Baseline	IV week	% Increase	P value	Baseline	IV week	% Decrease	P value	Baseline	IV week	% Decrease	P value
PlI	1.996	2.043	2.39%	0.025	1.808	1.163	35.67%	0.000	1.910	1.793	6.11%	0.000
GI	1.936	1.996	3.12%	0.011	1.727	1.187	31.26%	0.000	1.808	1.645	9.03%	0.000
BOP	73.280	76.290	4.11%	0.009	79.166	51.785	34.59%	0.000	78.214	73.571	5.94%	0.000
GCF	171.439	186.838	8.98%	0.003	200.899	103.106	48.68%	0.000	194.929	173.286	11.10%	0.000

$P<0.05$ was considered as statistically significant

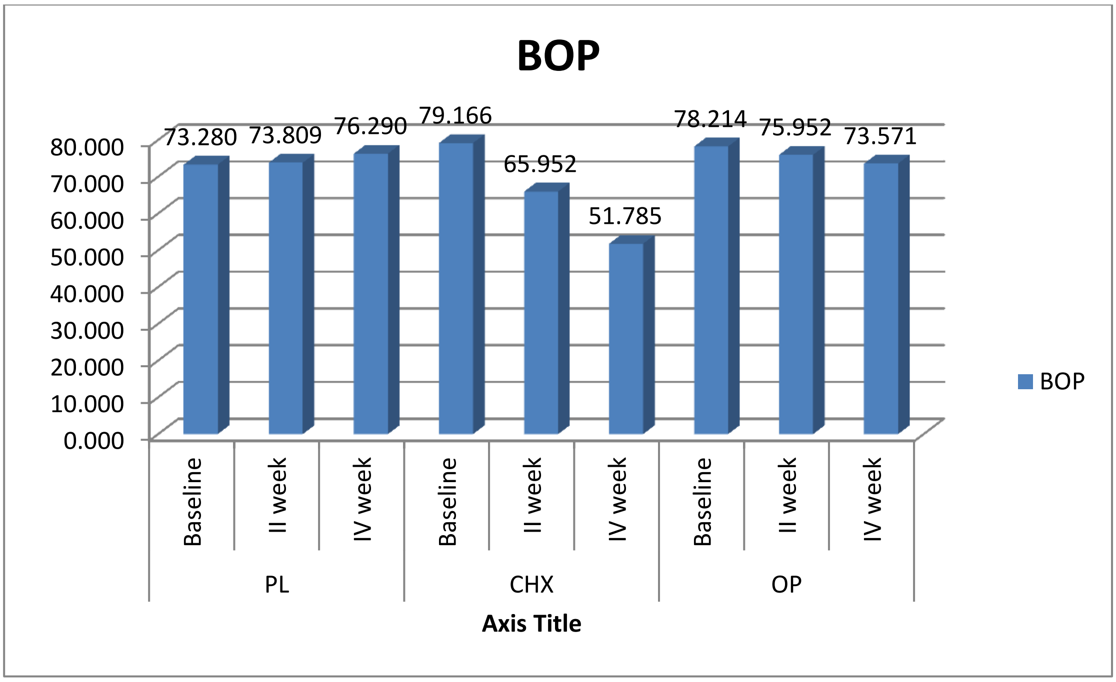
Graph. 2. Changes in PlI scores post-intervention



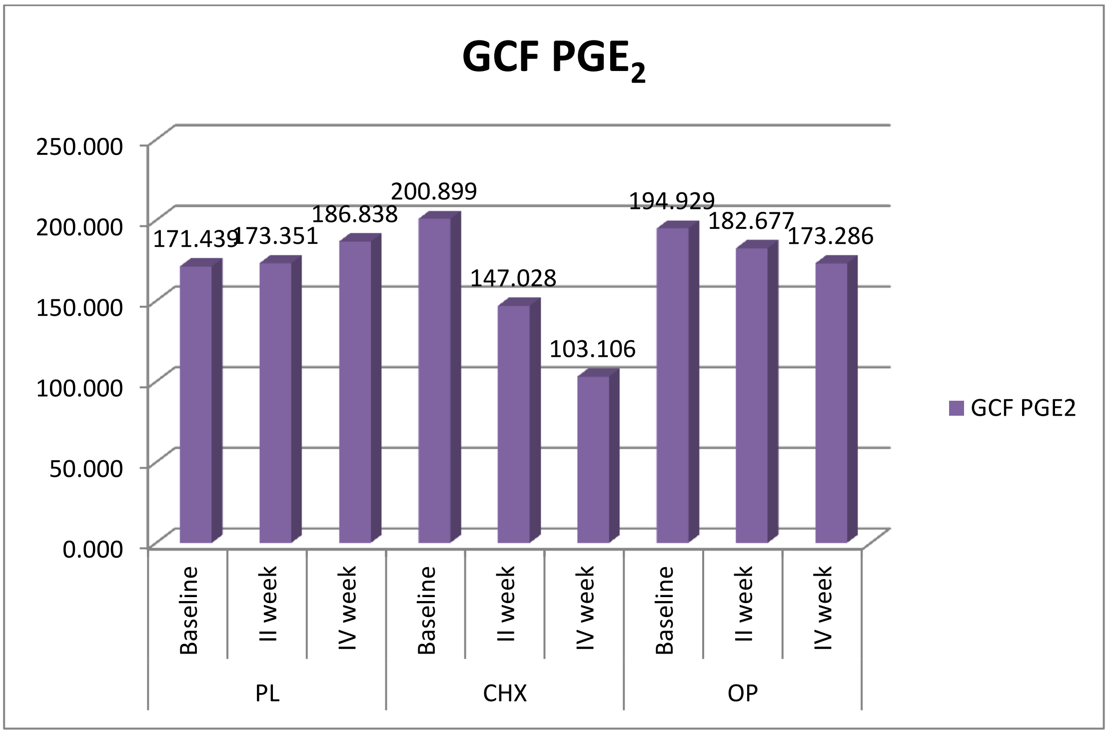
Graph. 3. Changes in GI scores post-intervention



Graph. 4. Percentage reduction in BOP post-intervention



Graph. 5. Changes in GCF PGE_2 levels post-intervention



Tab. 8. Comparison of CHX and OP (P value)

Parameters	Scores	t-test for Equality of Means P value Comparison
Reduction (Baseline to II week)	PlI	0.000
	GI	0.000
	BOP	0.000
	GCF PGE_2	0.000
Reduction (Baseline to IV week)	PlI	0.000
	GI	0.000
	BOP	0.000
	GCF PGE_2	0.000

(Independent samples ‘t’ test - $P<0.05$ was considered as statistically significant)

DISCUSSION

The aim of this study was to compare the efficacy of conventional mouthrinsing (CHX) with OP (Arimedadi Taila) in preventing plaque formation and reducing gingival inflammation. The changes in gingival inflammation were assessed using conventional clinical indices. The impact of these mouthrinses on crevicular PGE_2 levels during the interventional period (II week and IV week), was also assessed using ELISA technique.

CHX considered as the "Gold standard" mouthwash was used as the positive functional control in this study to assess and compare the effect of OP therapy in preventing plaque formation and reducing gingival inflammation. **Axelsson and Lindhe (1987),**[106] have shown that CHX mouthwash is effective in the reduction of plaque and gingivitis. **Menendez (2005),**[107] have shown that CHX is very effective against the formation of dental plaque. Many studies,[5,68,75,76,78] have been reported that CHX resulted in greater or equal reduction in PlI and GI scores compared to OP, but CHX has some disadvantages like tooth staining,[46,47,48,50,108] altered taste sensation[46] and allergic reactions.[46,55]

OP has been used extensively as a traditional Indian folk remedy for many years to prevent decay, oral malodor, bleeding gums, dryness of the throat, cracked lips and for strengthening teeth and gums.[63] It has the following advantages over CHX: no staining, no allergy, more cost effective, except for the extended duration of rinsing with the mouthwash.

Any oil like sesame oil, coconut oil, sunflower oil, groundnut oil, mustard oil or olive oil can be used for OP procedure. There were no published data available comparing the efficacy of different oils, which can be used for OP. OP with

Arimedadi Taila has been mentioned in the ancient ayurvedic literature, as a measure to prevent and cure numerous oral and systemic diseases. It is indicated in almost all diseases of oral cavity including stomatitis, glossitis, apthous ulcers, dental caries, pyorrhea, gingivitis, stains removal and hyperemia of gums.[6]

Till date, there were no published data available comparing the efficacy of Arimedadi Taila OP with CHX mouthrinsing in preventing plaque formation, reducing gingival inflammation and its biomarker in GCF. The role of PGE_2 as a biochemical marker of changes in gingival inflammation appears to have been less explored. Hence, this study was undertaken to compare the efficacy of Arimedadi Taila OP with CHX mouthrinsing in reducing plaque induced gingivitis and to assess the crevicular changes in PGE_2 level.

This study was a triple blind controlled clinical trial, wherein the participants, investigator and statistician were unaware of the intervention being administered. Patients attending the dental outpatient section at Department of Periodontics, at Sri Ramakrishna Dental College and Hospital, Coimbatore, who were diagnosed with gingivitis and who had satisfied the inclusion and exclusion criteria were included in the study.

In this study, 29 gingivitis patients were randomly allocated to any of the three interventional groups, which involved the use of CHX (positive functional control) or Arimedadi OP (Test) or PL mouthrinse containing mint flavored distilled water (negative control) for a period of 30 days. Along with their routine oral hygiene measures, these patients were instructed to use the mouthrinses assigned for them (without disclosing its nature), as prescribed by the manufacturer, twice daily. The principles of randomization and allocation concealment were followed in order to avoid bias.

In gingivitis patients, the tooth site showing greatest gingival inflammation and having greatest GI scores was used for GCF collection. PlI, GI, BOP scores were recorded at baseline, II week, IV week. Crevicular PGE_2 levels were analyzed using ELISA. The baseline sample characteristics, Baseline, post-intervention clinical parameters and PGE_2 levels and comparison of baseline and post-intervention findings have been summarized in Tab. 5, Tab. 6 and Tab. 7. respectively.

There was a significant decrease in PlI scores (Tab. 5, Fig. 15), following the use of CHX mouthrinses and OP for a period of 30 days. The mean reduction in PlI scores obtained with CHX mouthrinse was 16.79% at II week, 35.67% at IV week, 22.69% between II and IV week. This reduction in PlI scores was similar, when compared to that obtained by **Sikka et al (2011),**[109] who observed 16.49% and 32.17% reduction at II week and IV week. But this reduction was lesser, when compared to reduction of 20.71% and 44.97% at II week and IV week respectively, as reported by **Priya et al (2016).**[110]

The mean reduction in PlI scores obtained with OP at II week (3.07%), IV week (6.11%) and between II and IV week (3.13%) were significantly lesser, when compared with CHX mouthrinsing. It was lesser, when compared to 12.71% reduction at IV week, as reported by **Amit et al (2000)**[62], using sunflower OP and 13.13% reduction at VI week, as reported by **Saravanan et al (2013),**[111] using sesame OP on plaque induced gingivitis. Overall, from baseline to II week and IV week, when the reduction in PlI scores were compared for the CHX and OP groups, the difference was statistically significant (P=0). (Tab. 6.) CHX has anti-plaque activity which was found to be greater than OP and this could be attributed to the property of substantivity, which is the ability of an antimicrobial agent to persist within the oral cavity for long time. There exists no scientific explanation for anti-plaque action of Arimedadi Taila and whether the reduction in plaque scores observed with OP is purely due to physical action of an oily substance on tooth surface or by virtue of a biochemical action of its contents on periodontal microbiota, is yet to be ascertained.

In this context, it has been shown that saponification and emulsification could be the probable mechanisms through which sesame oil may exert its antibacterial and anti-plaque activity during OP.[63] Hence, similar studies need to be conducted to verify the physical and/or biochemical processes involved in the use of Arimedadi Taila.

In the present study, the difference between the mean GI scores (Tab. 5, Fig. 14) before and after CHX rinsing and OP was statistically significant. The mean reduction observed in GI scores following CHX rinses at II week, IV week and between II and IV week was 19.19%, 31.26%, and 14.94% respectively. This reduction in GI scores was slightly greater, when compared to that obtained by **Sikka et al (2011)**[109], who observed 11.61% and 24.18% reduction in GI scores, at II week and IV week respectively. But this reduction was lesser when compared to 28.08% and 46.06% reduction at II week and IV week, respectively as reported by **Priya et al (2016)** [110]**.**

In the OP group, this reduction at II week (3.67%), IV week (9.03%) and between II and IV week (5.56%) were significantly lesser when compared to CHX rinsing. It was lesser, when compared to reduction of 18.05% at II week and 33.33% at IV week as reported by **Amit et al (2007)**[62], using sunflower OP and 19.84% at IV week as reported by **Saravanan et al (2013)**[111] using sesame OP on plaque induced gingivitis. Overall, from baseline to II week and IV week, when the reduction in GI scores were compared for the CHX and OP groups, the difference was statistically significant (P=0). (Tab. 6.)

Significant reduction was observed in the number of gingival sites, which bled on probing (Tab. 5, Fig. 16) in both CHX and OP group. However, the reduction observed in CHX group at II week, IV week and between II and IV week was 16.69%, 34.59%, 21.48% respectively, as opposed to a reduction of 2.89%, 5.94% and 3.13% for the same, in the OP group. This reduction in BOP scores, observed in CHX group in our study was lesser, when compared to the reduction of 47.38%, as reported by **Biswas et al (2014)**[112] at IV week. Overall, from baseline to II week and IV week, when the reduction in BOP scores were compared for the CHX and OP groups, the difference was statistically significant (P=0). (Tab. 6.)

The results of this study also revealed that the use of CHX resulted in relatively greater reduction of crevicular PGE_2 levels at II week (26.81%), IV week (48.68%) and between II and IV week (29.87%), as compared to lesser reduction at II week (6.29%), IV week (11.10%) and between II and IV week (5.14%) in OP (Tab. 5, Fig. 17). Overall, from baseline to II week and IV week, when the reduction in GCF PGE_2 levels were compared for the CHX and OP groups, the difference was statistically significant (P=0). (Tab. 6.) To our knowledge, there were no published data available to compare the reduction in PGE_2 levels after mouthrinsing.

In the PL rinsing group, because of the continued plaque accumulation, the patients demonstrated increased PlI, GI and BOP scores (Tab. 5). At II week, there was an increase of 0.1% in PlI scores, 0.15% in GI scores, 0.72% in bleeding scores and 1.11% in PGE_2 levels, which were not significant. At IV week, there was significant increase of 2.39% in PlI scores, 3.12% in GI scores, 4.11% in BOP scores and 8.98% in crevicular PGE_2 levels.

These results revealed that there was an abrupt rise in crevicular PGE_2 levels at IV week, when compared to II week. These results are in accordance with **Heasman et al (1993)**[93] study on experimental gingivitis, where crevicular fluid PGE_2 levels remained fairly stable at baseline PGE_2 levels for the first 3 weeks, then rose sharply by 2.5 fold at 4 weeks. It may not be meaningful to directly compare the numerical data from other studies, due to the different populations and the different analytical techniques employed to measure crevicular PGE_2 concentrations, but trends can certainly be evaluated.

No serious adverse effects to any hard and soft tissues of the oral cavity were noticed, during this study. No patients reported of any mucosal changes, burning sensation in the oral cavity or the presence of ulcers or vesicles, which were suggestive of any allergic or hypersensitivity reactions. One patient of CHX group complained of altered taste sensation. Brownish staining was observed on the tooth surfaces of six patients of CHX group, while no staining reactions were observed in the participants of OP group. Five participants of OP group complained of abnormal taste sensation, associated with the use of Arimedadi Taila, which made it difficult for them to hold it in the oral cavity for a long time.

The results of the present study show that OP with Arimedadi Taila is not as effective as CHX in preventing plaque formation and resolving the clinical signs of gingival inflammation. There appears to be high inter-individual variability in crevicular PGE_2 levels. PGE_2 levels in GCF are found to be more sensitive to changes in gingival inflammation. Further longitudinal studies are required to assess the potential of PGE_2 as a marker to detect changes in gingival inflammation.

Limitations of our study are as follows: Microbiological assessment along with biochemical analysis, if considered, will add more validity to such studies. For better analysis of crevicular PGE_2 levels, more sensitive biochemical analytic method like chemiluminescent assay could have been performed, rather than ELISA. Longitudinal studies involving larger sample size are required for better assessment of changes in the clinical signs and crevicular PGE_2.

The following conclusions were drawn from this study –

- The anti-plaque and anti-gingivitis activity of CHX was superior to Arimedadi Taila, though there was significant reduction observed in both groups.
- Overall, from baseline to II week and IV week, when the reduction in PlI, GI, BOP scores were compared for the CHX and OP groups, the difference was statistically significant (P=0). (Tab. 6.)
- Following mouthrinsing, the crevicular PGE_2 levels were found to be decreased and this reduction was greater in CHX than OP group.
- In the PL group there was no anti-plaque and anti-gingivitis activity, which was in contrast to CHX and OP group.

SUMMARY AND CONCLUSION

OP with Arimedadi Taila has been mentioned in the ancient ayurvedic literature as a measure to prevent and cure numerous oral and systemic diseases. The present study was done to evaluate the anti-plaque and anti-gingivitis effects of OP with Arimedadi Taila as compared to mouthrinsing with Chlorhexidine in gingivitis patients. In this study, twenty nine gingivitis patients were randomly allocated to three chemical plaque interventional groups which involved the use of Chlorhexidine (positive functional control), Arimedadi Taila (Test) and PL containing Mint flavored distilled water (negative control) for a period of 30 days. Along with their routine oral hygiene measures, these patients were instructed to use the mouthrinses assigned for them, as prescribed twice daily.

The clinical parameters that were used for comparative assessment included Gingival and Plaque Index scores, and the number of sites with gingival bleeding before and after intervention at II week and IV week. The impact of these mouthrinses on GCF PGE_2 levels during the interventional period was also assessed using ELISA technique.

The following conclusions were drawn from this study –

- The anti-plaque and anti-gingivitis activity of CHX was superior to Arimedadi Taila, though there was significant reduction observed in both groups.
- Overall, from baseline to II week and IV week, when the reduction in PlI, GI, BOP and GCF PGE_2 values were compared for the CHX and OP groups, the difference was statistically significant (P=0).
- Following mouthrinsing, the crevicular PGE_2 levels were found to be decreased and this reduction was greater in CHX than OP group.

- In the PL group there was no anti-plaque and anti-gingivitis activity, which was in contrast to CHX and OP group.

A household remedy like OP which saves time and money and enhances oral and general health needs much more exploration. Extensive studies with larger samples, varying time periods and long follow up times should be carried out to establish the efficacy of OP therapy in prevention of plaque induced gingivitis

BIBLIOGRAPHY

1. Parameter on Plaque-Induced Gingivitis. American Academy of Periodontology. *J Periodontol* 2000;71:851-2.

2. van der Weijden F, Slot DE. Oral hygiene in the prevention of periodontal diseases: the evidence. *Periodontol 2000* 2011;55:104-123.

3. Mandel ID. Chemotherapeutic agents for controlling plaque and gingivitis. *J clin Periodontol* 1988;15:488-98.

4. Jones CG. Chlorhexidine: is it still the gold standard? *Periodontol 2000* 1997;15: 55-62.

5. Singh A, Purohit B. Tooth brushing, oil pulling and tissue regeneration: A review of holistic approaches to oral health *Journal of Ayurveda & Integrative Medicine.* 2011;2:64-8.

6. Madhusudhana R, Kranthi J, Srinivas R, Vallabh S. Arimedadi Taila, An Alternative Remedy in the Management of Dental Diseases. *International journal of innovative research & development* 2014;3:55-8

7. AlRowis R. Oral fluid-based biomarkers in periodontal disease-Part 2. Gingival crevicular fluid. *J Int Oral Health* 2014;6:126-35.

8. Jin LJ, Soder PO, Leung WK, Corbet EF, Samaranayake LP, Soder B. Granulocyte elastase activity and PGE_2 levels in gingival crevicular fluid in relation to the presence of subgingival periodontopathogens in subjects with untreated adult periodontitis. *J Clin Periodontol* 1999;26:531-40.

9. Loe H, Silness J. Periodontal Disease In Pregnancy. I. Prevalence And Severity. *Acta Odontol Scand* 1963;21:533-51.

10. Silness J, Loe H. Periodontal Disease In Pregnancy. Ii. Correlation Between Oral Hygiene And Periodontal Condition. *Acta Odontol Scand* 1964;22:121-35.

11. Mombelli A, van Oosten MA, Schurch E Jr, Land NP. The microbiota associated with successful or failing osseointegrated titanium implants. *Oral Microbiol Immunol* 1987;2:145-51.

12. Griffiths GS. Formation, collection and significance of gingival crevice fluid. *Periodontol 2000* 2003;31:32-42.

13. Osso D, Kanani N. Antiseptic mouth rinses: an update on comparative effectiveness, risks and recommendations. *J Dent Hyg* 2013;87:10-8.

14. Hebbar A, Keluskar V, Shetti A. Oil pulling unravelling path to mystic cure. *Journal of International Oral Health* 2010;2:11-4.

15. Waerhaug J. The gingival pocket; anatomy, pathology, deepening and elimination. *Odontol Tidskr* 1952;60:1-186.

16. Brill and Krasse. The Passage of Tissue Fluid into the Clinically Healthy Gingival Pocket. *Acta Odont. Scandinav 1958;16:231-45*.

17. Delima AJ, Van Dyke TE. Origin and function of the cellular components in gingival crevice fluid. *Periodontol 2000* 2003;31:55-76.

18. Egelberg J. Permeability of the dento-gingival blood vessels. IV. Effect of histamine on vessels in clinically healthy and chronically inflamed gingivae. *J Periodontal Res* 1966;1:297-302.

19. Bang JS, Cimasoni G. Total protein in human crevicular fluid. *J Dent Res* 1971;50:1683.

20. Golub LM, Kleinberg I. Gingival crevicular fluid: a new diagnostic aid in managing the periodontal patient. *Oral Sci Rev* 1976;8:49-61.

21. Alfano MC, Brownstein CN, Chasens AI, Kaslick RS. Passively generated increase in gingival crevicular fluid flow from human gingiva. *J Dent Res* 1976:55:1132.

22. Pashley DH. A mechanistic analysis of gingival fluid production. *J Periodontal Res* 1976;11:121-34.

23. Curtis MA, Griffiths GS, Price SJ, Coulthurst SK, Johnson NW. The total protein concentration of gingival crevicular fluid. Variation with sampling time and gingival inflammation. *J Clin Periodontol* 1988;15:628–32.

24. Darany DG, Beck FM, Walters JD.The relationship of gingival fluid leukocyte elastase activity to gingival fluid flow rate. *J Periodontol* 1992;63:743-7.

25. Lamster IB, Novak MJ. Host mediators in gingival crevicular fluid: implications for the pathogenesis of periodontal disease. *Crit Rev Oral Biol Med* 1992;3:31-60.

26. Cimasoni G. Crevicular Fluid Updated. *Monogr Oral Sci* 1983;12:1–152.

27. Sueda T, Bang J, Cimasoni G. Collection of gingival fluid for quantitative analysis. *J Dent Res* 1969;48:159.

28. Oppenheim FG. Preliminary observations on the presence and origin of serum albumin in human saliva. *Helv Odontol Acta* 1970;14:10–7.

29. Skapski H, Lehner T. A crevicular washing method for investigating immune components of crevicular fluid in man. *J Periodontal Res* 1976;11:19–24.

30. Bickel M, Cimasoni G. Reliability of volume measurements with the new Periotron 6000. *J Periodontal Res* 1984;19:313-6.

31. Koregol AC, More SP, Nainegali S, Kalburgi N, Verma S. Analysis of inorganic ions in gingival crevicular fluid as indicators of periodontal disease activity: A clinico-biochemical study. *Contemp Clin Dent* 2011;2:278-82.

32. Guentsch A, Kramesberger M, Sroka A, Pfister W, Potempa J, Eick S. Comparison of gingival crevicular fluid sampling methods in patients with severe chronic periodontitis. *J Periodontol* 2011;82:1051-60.

33. Mathur S, Mathur T, Srivastava R, Khatri R. Chlorhexidine: The Gold Standard in Chemical Plaque Control. *National Journal of Physiology, Pharmacy & Pharmacology* 2011;1:45-50

34. Chawner JA, Gilbert P. Interaction of the bisbiguanides Chlorhexidine and Alexidine with phospholipid vesicles: evidence for separate modes of action. *J Appl Bacteriol* 1989;66:253-8.

35. Davies A. The mode of action of Chlorhexidine. *J Periodontal Res Suppl* 1973;12:68-75.

36. Addy M. Chlorhexidine compared with other locally delivered antimicrobials. A short review. *J Clin Periodontol* 1986;13:957-64.

37. Roberts WR, Addy M. Comparison of the in vivo and in vitro antibacterial properties of antiseptic mouthrinses containing chlorhexidine, alexidine, cetyl pyridinium chloride and hexetidine. Relevance to mode of action. *J Clin Periodontol* 1981;8:295-310.

38. Schiott CR, Karring G, Karring T. Two years oral use of chlorhexidine in man. I. General design and clinical effects. J *Periodontal Res* 1976;11:135-44.

39. Hjeljord LG, Rolla G, Bonesvoll P. Chlorhexidine-protein interactions. *J Periodontal Res Suppl* 1973;12:11-6.

40. Lang NP, Ramseier-Grossmann K. Optimal dosage of Chlorhexidine digluconate in chemical plaque control when applied by the oral irrigator. *J Clin Periodontol* 1981;8:189-202.

41. Jenkins S, Addy M, Wade W. The mechanism of action of Chlorhexidine. A study of plaque growth on enamel inserts in vivo. *J Clin Periodontol* 1988;15:415-24.

42. Jenkins S, Addy M, Newcombe RG. Dose response of Chlorhexidine against plaque and comparison with triclosan. *J Clin Periodontol* 1994;21:250-5.

43. Loe H, Schiott CR. The effect of mouthrinses and topical application of Chlorhexidine on the development of dental plaque and gingivitis in man. *J Periodontal Res* 1970;5:79-83.

44. Cumming BR, Loe H. Optimal dosage and method of delivering Chlorhexidine solutions for the inhibition of dental plaque. *J Periodontal Res* 1973;8:57-62.

45. Brecx MC, Liechti T, Widmer J, Gehr P, Lang NP. Histological and clinical parameters of human gingiva following 3 weeks of chemical (Chlorhexidine) or mechanical plaque control. *J Clin Periodontol 1989;16:150-5.*

46. Flotra L, Gjermo P, Rolla G, Waerhaug J. A 4-month study on the effect of Chlorhexidine mouth washes on 50 soldiers. *Scand J Dent Res* 1972;80:10-7.

47. Lang NP, Hotz P, Graf H, Geering AH, Saxer UP, Sturzenberger OP *et al*. Effects of supervised chlorhexidine mouthrinses in children. A longitudinal clinical trial. *J Periodontal Res* 1982;17:101-11.

48. Grossman E, Meckel AH, Isaacs RL, Ferretti GA, Sturzenberger OP, Bollmer BW *et al*. A clinical comparison of antibacterial mouthrinses: effects of Chlorhexidine, phenolics, and sanguinarine on dental plaque and gingivitis. *J Periodontol* 1989;60: 435-440.

49. Loe H, Schiott CR, Karring G, Karring T. Two years oral use of chlorhexidine in man. I. General design and clinical effects. *J Periodontal Res* 1976;11:135-44.

50. Moran J, Addy M. The effect of surface adsorption and staining reactions on the antimicrobial properties of some cationic antiseptic mouthwashes. *J Periodontol* 1984;55:278-82.

51. Baker PJ, Coburn RA, Genco RJ, Evans RT. Structural determinants of activity of Chlorhexidine and alkyl bisbiguanides against the human oral flora. *J Dent Res* 1987;66:1099-106.

52. Quirynen M, Mongardini C, de Soete M, Pauwels M, Coucke W, van Eldere J *et al.* The role of chlorhexidine in the one-stage full-mouth disinfection treatment of

patients with advanced adult periodontitis. Long-term clinical and microbiological observations. *J Clin Periodontol* 2000;27:578-89.

53. Sekino S, Ramberg P, Uzel NG, Socransky S, Lindhe J. Effect of various chlorhexidine regimens on salivary bacteria and de novo plaque formation. *J Clin Periodontol* 2003;30:919-25.

54. Karpinski TM, Szkaradkiewicz AK. Chlorhexidine-pharmaco-biological activity and application. *J Clin Periodontol* 2003;30:919-25.

55. Sharma A, Chopra H. Chlorhexidine urticaria: a rare occurrence with a common mouthwash. *Indian J Dent Res* 2009;20:377-9.

56. Berchier CE, Slot DE, Van der Weijden GA. The efficacy of 0.12% chlorhexidine mouthrinse compared with 0.2% on plaque accumulation and periodontal parameters: a systematic review. *J Clin Periodontol* 2010;37:829–39

57. van Maanen-Schakel NW, Slot DE, Bakker EW, Van der Weijden GA. The effect of an oxygenating agent on chlorhexidine-induced extrinsic tooth staining: a systematic review. *Int J Dent Hyg* 2012;10:198-208.

58. Pedrazzi V, Escobar EC, Cortelli JR, Haas AN, Andrade AK, Pannuti CM *et al.* Antimicrobial mouthrinse use as an adjunct method in peri-implant biofilm control. *Braz Oral Res* 2014;28:1-9

59. Slot DE, Berchier CE, Addy M, Van der Velden U, Van der Weijden GA. The efficacy of chlorhexidine dentifrice or gel on plaque, clinical parameters of gingival inflammation and tooth discoloration: a systematic review. *Int J Dent Hyg* 2014;12:25.

60. Jose A, Butler A, Payne D, Maclure R, Rimmer P, Bosma ML. A randomised clinical study to evaluate the efficacy of alcohol-free or alcohol-containing mouthrinses with chlorhexidine on gingival bleeding. *Br Dent J* 2015;219:125-30.

61. Marrelli M, Amantea M, Tatullo M. A comparative, randomized, controlled study on clinical efficacy and dental staining reduction of a mouthwash containing Chlorhexidine 0.20% and Anti Discoloration System (ADS). *Ann Stomatol (Roma)* 2015;35-42.

62. Amith HV, Ankola AV, Nagesh L. Effect.Effect of Oil Pulling on Plaque and Gingivitis, *J Oral Health Comm Dent* 2007;1:12-8.

63. Asokan S, Rathinasamy TK, Inbamani N, Menon T, Kumar SS, Emmadi P. Mechanism of oil-pulling therapy - in vitro study. *Indian J Dent Res* 2011;22:34-7.

64. Amruthesh S. Dentistry and Ayurveda--III (basics-ama, immunity, ojas, rasas, etiopathogenesis and prevention*). Indian J Dent Res* 2007;183:112-9.

65. Abraham S, Kumar MS, Sehgal PK, Nitish S, Jayakumar ND. Evaluation of the inhibitory effect of triphala on PMN-type matrix metalloproteinase (MMP-9). *J Periodontol* 2005;76:497-502.

66. Yun JH, Kim CS, Cho KS, Chai JK, Kim CK, Choi SH. (-)-Epigallocatechin gallate induces apoptosis, via caspase activation, in osteoclasts differentiated from RAW 264.7 cells. *J Periodontal Res* 2007;42:212-8.

67. Asokan S, Rathan J, Muthu MS, Rathna PV, Emmadi P. Effect of oil pulling on Streptococcus mutans count in plaque and saliva using Dentocult SM Strip mutans

test: a randomized, controlled, triple-blind study. *J Indian Soc Pedod Prev Dent* 2008;26:12-7.

68. Asokan S, Emmadi P, Chamundeswari R. Effect of oil pulling on plaque induced gingivitis: a randomized, controlled, triple-blind study. *Indian J Dent Res* 2009;20: 47-51.

69. Surathu N, Kurumathur AV. Traditional therapies in the management of periodontal disease in India and China. *Periodontol 2000* 2011;56:14-24.

70. Kukreja B J, Dodwad V. Herbal mouthwashes - A gift of nature, *International Journal of Pharma and Bio Sciences* 2012;3:46-52.

71. Hosokawa Y, Hosokawa I, Ozaki K, Nakanishi T, Nakae H, Matsuo T. Tea polyphenols inhibit IL-6 production in tumor necrosis factor superfamily 14-stimulated human gingival fibroblasts. *Mol Nutr Food Res* 2010;54:151-8.

72. Tamura M, Saito H, Kikuchi K, Ishigami T, Toyama Y, Takami M *et al*. Antimicrobial activity of Gel-entrapped catechins toward oral microorganisms. *Biol Pharm Bull 2011;*34:638-43.

73. Dani N, Kale T, Beldar A, Raghavan M, Thakkar P. Oil pulling as an adjunct to scaling and root planing: A Clinico-Microbial study. *International Journal of Pharmaceutical Science Invention 2015;*4:38-44.

74. Bajaj N, Tandon S. The effect of Triphala and Chlorhexidine mouthwash on dental plaque, gingival inflammation, and microbial growth. *Int J Ayurveda Res* 2011;2:29-36.

75. Shetty S, Pillai S, Sridharan S, Satyanarayana A, Rahul A. Comparative Efficacy of Chlorhexidine and a Herbal Mouth Rinse in Patients with Gingival Inflammation -

A Clinical & Microbiologic Study. *Asian Journal of Pharmaceutical Technology & Innovation* 2013;1;1-8.

76. Naiktari RS, Gaonkar P, Gurav AN, Khiste SV. A randomized clinical trial to evaluate and compare the efficacy of triphala mouthwash with 0.2% chlorhexidine in hospitalized patients with periodontal diseases. *J Periodontal Implant Sci* 2014;44: 134-40

77. Lakade LS, Shah P, Shirol D. Comparison of antimicrobial efficacy of chlorhexidine and combination mouth rinse in reducing the Mutans streptococcus count in plaque. *J Indian Soc Pedod Prev Dent* 2014;32:91-6.

78. Gupta D, Gupta RK, Bhaskar DJ, Gupta V. Comparative evaluation of terminalia chebula extract mouthwash and chlorhexidine mouthwash on plaque and gingival inflammation - 4-week randomised control trial. *Oral Health Prev Dent* 2015;13: 5-12.

79. Elattar T M A. Prostaglandins: Physiology, biochemistry, pharmacology and clinical applications. *Journal of oral pathology* 1978;7:175-207

80. Kalinski P. Regulation of Immune Responses by Prostaglandin E 2. *J Immunol* 2012;188:21-8

81. Noguchi K, Ishikawa I. The roles of cyclooxygenase-2 and prostaglandin E2 in periodontal disease. *Periodontol 2000* 2007;43:85-101

82. Takayama K, Garcia-Cardena G, Sukhova GK, Comander J, Gimbrone MA Jr, Libby P. Prostaglandin E2 suppresses chemokine production in human macrophages through the EP4 receptor. *J Biol Chem* 2002;277:44147-54.

83. Ricciotti E, FitzGerald GA. Prostaglandins and inflammation. *Arterioscler Thromb Vasc Biol* 2011;31:986-1000.

84. Williams TJ, Peck MJ. Role of prostaglandin-mediated vasodilation in inflammation. *Nature* 1977;8:530-2.

85. Gemmell E, Marshall RI, Seymour GJ. Cytokines and prostaglandins in immune homeostasis and tissue destruction in periodontal disease. *Periodontol 2000;* 1997;14:112-43.

86. Offenbacher S, Odle BM, Gray RC, Van Dyke TE. Crevicular fluid prostaglandin E levels as a measure of the periodontal disease status of adult and juvenile periodontitis patients. *J Periodontal Res* 1984;19:1-13.

87. Offenbacher S, Odle BM, Van Dyke TE. The use of crevicular fluid PGE_2 levels as a predictor of periodontal attachment loss. *J Periodont Res* 1986;21:101-12.

88. Nakashima K, Roehrich N, Cimasoni G. Osteocalcin, prostaglandin E2 and alkaline phosphatase in gingival crevicular fluid: their relations to periodontal status. *J Clin Periodontol* 1994;21:327-33.

89. Ebersole JL, Singer RE, Steffensen B, Filloon T, Kornman KS. Inflammatory mediators and immunoglobulins in GCF from healthy, gingivitisand periodontitis sites. *J Periodontal Res* 1993;28:543-6.

90. Offenbacher S, Heasman PA, Collins JG. Modulation of host PGE2 secretion as a determinant of periodontal disease expression. *J Periodontol* 1993;64:432-44.

91. Offenbacher S, Odle BM, Braswell LD. Changes in cyclooxygenase metabolites in experimental Periodontitis in Macaca mulatto. *J Periodont Res* 1989;24:63-74.

92. Ohm K, Albers Von H-K, Lisboa BP. Measurement of 8 Prostaglandins in human gingival and periodontal disease using high pressure liquid chromatography and radioimmunoassay. *J Periodont Res* 1984;33:235-49.

93. Heasman PA, Collins JG, Offenbacher S. Changes in crevicular fluid levels of interleukin-1 beta,leukotriene B4, prostaglandin E2, thromboxane B2 and tumour necrosis factor alpha in experimental gingivitis in humans. *J Periodontal Res* 1993;28:241-7.

94. Preshaw PM, Heasman PA: Prostaglandin E_2 concentrations in gingival crevicular fluid: observations in untreated chronic periodontitis. *J Clin Periodontol* 2002;29: 15-20.

95. Downie J, Poyser N L, Wunderlich M. Levels of Prostaglandins in human endometrium during the normal menstrual cycle. *J physiol* 1974;236:465-72

96. Mendieta CF, Reeve CM, Romero JC. Biosynthesis of prostaglandins in gingiva of patients with chronic periodontitis. *J Periodontol* 1984;56:44-7.

97. Alexander DC, Martin JC, King PJ, Powell JR, Caves J, Cohen ME. Interleukin-1 beta, prostaglandin $E_{2,}$ and immunoglobulin G subclasses in gingival crevicular fluid in patients undergoing periodontal therapy. *J Periodontol* 1996;67:755-62.

98. Noguchi K, Shitashige M, Ishikawa I. Involvement of cyclooxygenase-2 in interleukin-1alpha-induced prostaglandin production by human periodontal ligament cells. *J Periodontol* 1999;70:902-8.

99. Morton RS, Dongari-Bagtzoglou AI. Cyclooxygenase-2 is upregulated in inflamed gingival tissues. *J Periodontol* 2001;72:461-9.

100. Rausch-Fan X, Ulm C, Jensen-Jarolim E, Schedle A, Boltz-Nitulescu G, Rausch WD *et al.* Interleukin-1beta-induced prostaglandin E2 production by human gingival fibroblasts is upregulated by glycine. *J Periodontol* 2005;76:1182-8.

101. Siegel B, Weihe E, Bette M, Nusing RM, Flores-de-Jacoby L, Mengel R. The effect of age on prostaglandin-synthesizing enzymes in the development of gingivitis. *J Periodontal Res* 2007;42:259-66.

102. Becerik S, Ozcaka O, Nalbantsoy A, Atilla G, Celec P, Behuliak M *et al*. Effects of menstrual cycle on periodontal health and gingival crevicular fluid markers. *J Periodontol* 2010;81:673-81.

103. Lai YL, Wu CY, Lee YY, Chang HW, Liu TY, Hung SL. Stimulatory effects of areca nut extracts on prostaglandin E2 production by human polymorphonuclear leukocytes. *J Periodontol* 2010;81:758-66.

104. Offenbacher S. Changes in gingival crevicular fluid inflammatory mediator levels during the induction and resolution of experimental gingivitis in humans. *J Clin Periodontol* 2010;37:324-33

105. Kumar KA, Reddy NR, Babu M, Kumar PM, Venkat Subba Reddy VS, Chavan CV. Estimation of prostaglandin E_2 levels in gingival crevicular fluid in periodontal health, disease and after treatment. *Contemp Clin Dent* 2013;4:303–6.

106. Axelsson P, Lindhe J. Efficacy of mouthrinses in inhibiting dental plaque and gingivitis in man. *J Clin Periodontol* 1987;14:205-12.

107. Menendez A, Li F, Michalek SM, Kirk K, Makhija SK, Childers NK. Comparative analysis of the antibacterial effects of combined mouthrinses on Streptococcus mutans. *Oral Microbiol Immunol* 2005;20:31-4.

108. Rahman B, Alkawas1S, Al Zubaidi EA, Adel OI, Hawas N. Comparative antiplaque and antigingivitis effectiveness of tea tree oil mouthwash and a cetylpyridinium chloride mouthwash: A randomized controlled crossover study. *Contemporary Clinical Dentistry* 2014;5:466-70.

109. Sikka G, Dodwad V, Chandrashekar KT. Comparative Anti-plaque and Anti-gingivitis Efficacy of Two Commercially Available Mouthwashes - 4 Weeks Clinical Study. *Journal of Oral Health Community Dentistry* 2011;5:110-2.

110. Priya BM, Galgali SR. Comparison of amine fluoride and chlorhexidine mouth rinses in the control of plaque and gingivitis--a randomized controlled clinical trial. *Indian J Dent Res* 2015;26:57-62.

111. Saravanan D, Ramkumar S, Vineetha K. Effect of Oil Pulling with sesame Oil on Plaque-induced Gingivitis: A microbiological Study. *Journal of Orofacial Research* 2013;3:175-80.

112. Biswas G, Anup N, Acharya S, Kumawat H, Vishnani P, Tambi S. Evaluation of the Efficacy of 0.2% Chlorhexidine versus Herbal Oral Rinse on Plaque Induced Gingivitis- A Randomized Clinical Trail. *IOSR - Journal of Nursing and Health Science* 1940;3:58-63.